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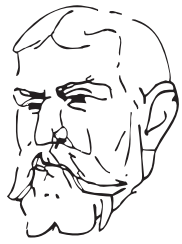

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Biegański

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CONTENTS

ORIGINAL ARTICLE

VASCULAR-PLATELET HEMOSTASIS OF INJURED PATIENTS: PROSPECTIVE OBSERVATIONAL STUDY

DOI: 10.36740/WLek202307101

Mariana Vyshynska, Khrystyna Dutko
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ABSTRACT

The aim: We study vascular-platelet hemostasis peculiarities in patients with severe trauma.

Materials and methods: We included 50 patients, who were divided into control (n=15) and study (n=35) groups. The control group included patients without traumatic injuries, study group – patients with severe trauma. The study group was divided into the I subgroup (patients received 1 g tranexamic acid IV at the prehospital stage), and the II subgroup (1 g tranexamic acid IV after hospital admission).

Results: The main changes in the I subgroup started on the 3rd day, while in the II subgroup – on the 1st day. Patients of both subgroups on the 1st and 3rd days had a normal number of platelets in venous blood, however, on the 3rd day, there was a decreasing level of discocytes whereas the level of discoechinocytes, spherocytes, spheroechinocytes, and the sum of active forms of platelets were increased in comparison with the control group ($p<0.05$).

Conclusions: The changes in vascular-platelet hemostasis in patients appeared in the I subgroup on the 3rd day, while in the II subgroup – on the 1st day. For the I subgroup was the decreasing level of discocytes, whereas the level of discoechinocytes, spherocytes, spheroechinocytes, and the sum of active forms of platelets were increased. For the II subgroup on the 1st day, there was an increasing sum of active forms of platelets, on the 3rd day – the level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes, and the sum of active forms of platelets were increased.

KEY WORDS: coagulopathy, severe trauma, platelets activation, vascular-platelet hemostasis

Wiad Lek. 2023;76(7):1511-1516

INTRODUCTION

Traumatic injuries are the third-largest cause of mortality in the general population in Europe and the leading cause of mortality in young patients [1]. Coagulopathy is a common complication after injury and develops independently from iatrogenic, hypothermic, and dilutional causes [2]. Combined tissue injury and shock result in hemostatic failure, which has been identified as a multidimensional molecular, physiologic and clinical disorder termed trauma-induced coagulopathy (TIC) [3]. Blood loss associated with acute traumatic coagulopathy is a leading cause of death following injury [4]. TIC is associated with increased early transfusion requirements, the development of organ failure, and mortality [5]. Owing to the innate crosstalk between coagulation and inflammation, there are widespread adverse downstream inflammatory and immune consequences associated with early trauma coagulopathies, including organ dysfunction and thromboembolic complications [4].

Although, all modern research is devoted to coagulation factors, platelets play an important role in thrombus formation. Careful assessment of platelet dysfunction is hampered by the technical complexity of existing techniques for studying platelet function under conditions that would meet the conditions of the human body. It is also unclear how to use and interpret vascular hemostasis in patients with trauma coagulopathy. Understanding the biology of TIC is of utmost importance, as it is often responsible for uncontrolled bleeding, organ failure, thromboembolic complications, and death.

THE AIM

The aim was to study vascular-platelet hemostasis peculiarities in patients with severe trauma, to single out these changes and find out primary hemostasis pathophysiology specifics in case of trauma-induced coagulopathy. The hypothesis was that morphological changes would not be detected in the first day after receiving the injury in patients with severe trauma.

MATERIALS AND METHODS

In January 2021 – October 2021 it was conducted the prospective observation study at the Department of

Anesthesiology and Intensive Care, Danylo Halytsky Lviv National Medical University; Department of Anesthesiology and Intensive Care at the Municipal Non-Profit Enterprise "8th City Clinical Hospital of Lviv". The reserch was conducted in accordance with the requirements of the Helsinki Declaration of the World Medical Association, the Council of Europe Convention on Human Rights. The reserch was approved by the Bioethics Commission of Danylo Halytsky Lviv National Medical University, protocol No.7, September 20, 2021. Patients were included to the reserch after signing an informed consent.

Criteria for inclusion in the study were: consent of the patient or his legal representatives to participate in the study, polytrauma, administration of tranexamic acid on the first day after injury. Criteria for exclusion from the study were: the patient's refusal to participate in the study, medical history of congenital pathology of the hemostasis system, the agonal state of the patient.

Study included 50 patients aged 19 to 55 years. All patients were divided into 2 groups. First, the control group, included 15 patients of the therapeutic department with trauma, without preconditions for changes and in the absence of laboratory-confirmed disorders in the hemostasis system. Second, the study group included 35 patients with a diagnosis of "polytrauma" (severe trauma), who were admitted to the Hospital Anesthesiology and Intensive Care department. Next, patients with polytrauma were divided into two subgroups, depending on they received tranexamic acid in the prehospital stage or after hospitalization.

The severity of the patients' condition was assessed by the Injury Severity Score (ISS). We studied: platelet count and hematocrit; indicators of vascular-platelet hemostasis (intravascular platelet activation, platelet aggregation induced by adrenaline and adenosine diphosphate) were determined by turbinometric method, using a phase contrast microscope. The main stages of the study were: prehospital stage (1st hour after injury), 1st, 3rd, 5th day after admission. Also we analyzed the volume of crystalloids and colloids at all stages of the study, the use of tranexamic acid in the prehospital stage and on the 1st day after admission to treatment, the volume of fresh frozen plasma and erythrocyte mass on the 1st, 3rd, 5th that day from the moment of admission to treatment. The presence of disseminated intravascular coagulation syndrome was performed by the ISTH Scale (International Society on Thrombosis and Haemostasis Scale) using a medical calculator [7]. We used Kruskal-Wallis test. The obtained data were checked for the normality of the distribution, they did not correspond to it, therefore the description was carried out using statistical indicators for data that do not correspond to the normal distribution.

RESULTS

The severity of patient`s state was assessed by the ISS scale and we found that, less than 9 points were not received by any of the patientsof the main group, 9–15 points were evaluated by 17 % of patients with polytrauma, 16–25 points were 71 % patients, more than 25 points –12 % of patients. The severity of patients` state is given in Table I-III.

We found no significant differences in age, body weight, body mass index (BMI) between patients in control and study groups.

Changes of vascular-platelet hemostasis in patients with severe trauma (study group) (tabl 3) at all stages of the study had significant differences from those in the control group. Main changes in I subgroup started on 3rd day, while in II subgroup – on 1st day. Patients of both subgroups on 1st and 3rd days had normal number of platelets in venous blood, however, on 3rd day there were decreasing level of discocytes, whereas level of discoechinocytes, spherocytes, spheroechinocytes and sum of active forms of platelets were increased. On the other hand, in II subgroup against the background of normal count of platelets on 1st day there was increasing sum of active forms of platelets, on 3rd day – level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes and the sum of active forms of platelets were increased.

After analyzing the frequency of transfusion of erythrocyte mass and fresh-frozen plasma, it was found that transfusion throughout treatment was performed in 15 cases out of 25, which was 60 % of all cases of polytrauma. In 9 cases out of 25 –transfusion was performed on the first day, which is 36 %. The ratio of erythrocyte mass to fresh-frozen plasma volume on the first day of treatment was 1:1, erythrocyte mass was 475±35 ml, and fresh-frozen plasma volume was 458±15 ml. Analyzing the literature, it should be noted that coagulopathy is one of the most common complications of polytrauma. The reason is a combination of shock caused by bleeding, tissue damage–associated with the regulation of thrombomodulin, the generation of throm-bin-thrombomodulin complex, activation of anticoagu-lant and fibrinolytic systems [5]. Disorders of hemostasis after injury are associated not only with iatrogenic causes –impaired thrombin production and platelet function due to hypothermia, acidosis and dilution coagulopathy, and with modern hemostatic treatments dilution coagulopathy is rare. To induce clinical manifestations of trauma-induced coagulopathy, the trigger is tissuedamage (as a consequence, activation of the coagulation cascade, thrombin production and stimulation of anticoagulant pathways), which must be combined with tissue hypoperfusion (i is believed that

Table I. Assessment of the severity of patients by Injury Severity Score (ISS)

ISS	Study group (n, %)
<9 points	-
9-15 points	6 (17%)
16-25 points	25 (71%)
> 25 points	4 (12%)

Table II. Anthropometric characteristics of patients (Me [Q1; Q3])

Parameters	Control group (n=15)	Study group (n=35)
Age, years	39.3 [29.1; 43.5]	36.6 [31.3; 44.8]
Body weight, kg	74.6 [63.4; 81.9]	74.9 [68.2; 84.5]
BMI, kg / m^2	24.9 [19.8; 27.4]	26.1 [21.3; 30.1]

Table III. Indicators of intravascular platelet activation (Me [Q1; Q3])

Indicators	Control group (n=15)	Study group (n=35)					
		1st day (d1)		3rd day (d3)		5th day (d5)	
		Subgroup					
		I (n=6)	II (n=29)	I (n=6)	II (n=29)	I (n=29)	II (n=29)
The number of platelets in the venous blood, 10^9/ l	200.7 [183; 213]	169 [141; 198]*	168 [136; 184]	161 [136; 184]*	140 [122; 151.4]	173.3 [151; 191]*	150 [133; 156]
Discocytes, %	81.4 [73; 93]*	81.6 [69.1; 94.5]	77.1 [63; 85]*	76.5 [65.3; 85.2]	74.5 [64; 83]*	82.2 [67.1; 93.4]	86.2 [67; 93]*
Discoechinocytes, %	8.4 [4.8; 8.8]	17.0 [12.1; 28.5]	18.2 [13.7; 24.1]	18.4 [11.3; 28.5]	18.8 [13.2; 23.9]*	12.1 [6.3; 24.4]	12.7 [8.8; 16.4]
Spherocytes, %	5.6 [3.8; 8.5]	1.6 [1.1; 6.5]	1.7 [1.1; 3.6]*	3,2 [2.3; 6.2]	3,6 [2.2; 6.1]*	3.0 [2.3; 7.7]*	3.2 [2.3; 8.6]*
Spheroechinocytes, %	3.0 [2.3; 5.6]	4.5 [2.1; 6.7]	4.6 [2.3; 8.6]	2.8 [1.3; 6.6]	3 .1 [1.3; 5.2]	1.1 [0.4; 5.8]*	1.5 [1.1; 3.6]*
Bipolar forms, %	0	0	0	0	0	0	0
The sum of the active forms of platelets, %	12.7 [10.1; 16.5]	17.3 [12.1; 25.3]	23 [21.1; 28.7]*	22.2 [15.1; 29.5]	24.2 [21.2; 30.4]*	19.3 [13.2; 27.3]	19.3 [11.2; 26.4]*
The number of platelets involved in the aggregates, %	14 [10; 18]*	3.2 [1.2; 6.4]*	3.4 [1.2; 6.5]	6.5 [4.3; 9.4]*	6.6 [3.2; 9.4]**	7.6 [4.2; 11.4]*	7.5 [4.4; 9.5]**

Note: * – $p<0.05$ compared with the control group of patients

this leads to contact, induced expression of thrombomodulin and endothelial protein C on the surface of the endothelium to activate protein C) [3]. Identifying distinct pathways implicated in TIC is critical to tailoring targeted resuscitation practices for improved outcomes after injury, and the practice of transfusing platelets in equal ratios to blood and plasma has become standard of care regardless of platelet count because platelets are known to play a pivotal role in normal coagulation and maintenance of endothelial integrity. The effect of trauma and shock on vascu-lar platelet hemostasis remains unexplored [6], platelets in hemostasis in polytrauma play a critical role, and their low level predicts mortality [7]. Kornblith L. Z. et. al. demonstrated that the contribution of platelets to the strength of clots in trauma is higher than the contribution of fibrinogen.

In patients with trauma, decreased platelet responsiveness to ADP secondary to downregulation of platelet $P2Y_{12}$ receptor (it is a G-protein coupled receptor that binds adenosine diphosphate (ADP). Consequently down regulation of this receptor or antagonist blockade inhibits ADP-mediated platelet aggregation. Thrombin when bound to TM increases anticoagulant activity through activation of protein C. But thrombin-TM interactions also promote antifibrinolytic activity by thrombin-mediated activation of TAFI (thrombin-activatable fibrinolysis inhibitor). Activated TAFI interferes with plasminogen binding to fibrin clots, which is required for plasminogen conversion to plasmin by plasminogen activators [8]. Anemia caused by bleeding or dilution due to fluid resuscitation may also affect platelet adhesion.

DISCUSSION

Thus, our research confirmed the significant the role of platelets in the pathogenesis of post-traumatic coagulopathy. It was established that the level of discocytes decreases, the level of discoechinocytes, spherocytes, spheroechinocytes and the amount of active forms of platelets increase in I subgroup on 3rd day. For II subgroup on 1st day there was increasing sum of active forms of platelets, on 3rd day – level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes and the sum of active forms of platelets were increased. There is a little knowlagge about ascular-platelet hemostasis in trauma patients, but a similar study was conducted by George MJ et al. (2020), measuring the force of platelet contraction (as a platelet contraction test). Their results show that platelet hyperfunction is observed in trauma patients who survive, and platelet dysfunction in patients who died. [9]. Hofer V et al. (2019) was analyzed platelet function on Platelet Function Analyzer (PFA 100) with adenosine diphosphate (ADP) and epinephrine as activation factors. They research discovered, that approximately one quarter to one third of primarily admitted trauma patients without long-term anticoagulation medication showed a delayed platelet activation in the PFA-100 test. By considering all trauma patients an even higher rate can be expected [10]. The contribution of platelet disorders to trauma-induced coagulopathy is not sufficiently understood and these aspects should be the subject of further research and our research try to reveal the dark side of the issue of platelet disorders in severe trauma patients.

It is well known that vascular-platelet hemostasis begins with reflex spasm of arterioles, due to the release of platelets of catecholamines and seretonin, followed by adhesion and aggregation, synthesis, accumulation and secretion during activation of substances and the formation of the final platelet thrombus. Even before contact with unmasked collagen, platelets begin to change their form from discoid (D) to activated cells of discoechinocytes (DE), spherocytes (S) and/or spheroehfnocytes (SE) [11]. DE differs from D by the presence of single and short processes, which appear after activation within the first second, and are the result of internal pressure on the plasma membrane of actin filaments. S is a more spherical cell, and SE is a spherical cell with a larger number of long processes. During the contact phase, the processes of activated platelets interact with the elements of the basement membrane of the vascular wall. The direct contact of platelet processes with collagen and the contact of platelets with collagen through Willebrand factor are important. On the surface of D, under the influence of collagen, factor XI is activated without changing its form. Thus D, which is incapable of direct development of aggregation, secretion, refraction, can be subjected to receptor membrane activation and, accordingly, the altered forms are caused by substances for which there are specific receptors on the platelet membrane, thrombin, collagen, adenosine phosphate (ADP), serotonin and other agonists. The hemostatic activity of platelets appears with the conversion of D to DE, and DE is capable of both pronounced adhesion and aggregation, due to exposure in this phase on the plasma membrane of fibrinogen receptors. Platelet aggregation is caused by the appearance of substances of aggregates of platelet or non-platelet origin. The most important are ADP, which is released from damaged cells of the vascular wall, hemolyzed erythrocytes, platelets; thromboxane A, adrenaline, serotonin, platelet aggregation factor, thrombin. The appearance of appendages also promotes aggregation, increasing the likelihood of platelet collisions, which is necessary for this process. In the future, with the formation of a significant amount of SE, the aggregation activity decreases slightly and begins to develop refractoriness of the cell, which is most pronounced in SE. In traumatized patients, platelets lose aggregative function as part of acute coagulopathy, which develops within minutes of injury, increases bleeding, and has a major impact on the risk of multiple organ failure and mortality. The decrease in the ability of platelets to aggregation occurs in parallel with the increase in their procoagulant function.

The main number of studies of primary hemostasis were performed using thromboelastography. Although the question of early platelet dysfunction in coagulopathy due to polytrauma remains unclear, a number of studies suggest that attenuation of platelet stimulation to adenosine diphosphate agonism may be secondary. Fecher A, et al. were assessed platelet function in thrombus composition and stability [12] and found that platelet dysfunction is detected after serious injury and before significant fluid or blood injections. In our research we studied the functional ability of platelets induced by epinephrine and ADP, which can be extrapolated to conditions similar to platelet activation in a trauma patient.

Stalker et al. (2013), in a mouse model [13], described a hemostatic plug consisting of a "core" region of tightly packed platelets surrounded by an outer "shell" region of more loosely packed platelets. Whereas platelets in the core region are isolated from the plasma and exposed to high levels of thrombin and collagen, the shell region is exposed to circulating plasma and grows by platelet-ADP interactions. Some degree of inhibition along the ADP pathway may therefore be normal after

trauma to counterbalance widespread activation of procoagulant mechanisms; another finding supporting this hypothesis is that platelet inhibition along the ADP pathway increases clot sensitivity to tPA-mediated fibrinolysis. Alternatively, platelet assays may inherently select for more dysfunctional platelets because functional platelets were removed from the circulation and incorporated into clots [14].

In most patients with trauma-induced coagulopathy, the number of platelets in the blood remains activated, but paradoxically impaired aggregation reactions exvivo. This phenomenon is de-scribed as "platelet depletion" caused by trauma and shock [15]. This phenomenon driven by endothelial release of TF, platelet activating factor and vWF [16]. In our research we learned pathophysiological changes of vascular-platelet hemostasis in patients with severe trauma, our future research will be able to show how these changes affect the treatment of this patients.

STUDY LIMITATIONS

The presented fragment of the study aimed to examine whether the indicators of vascu-lar-platelet and coagulation hemostasi in patients with trauma, compared with almost healthy individuals of the same age. In addition, the study was conducted on a very small sample size, which is insufficient to substantiate the role of vascular platelet hemostasis as a marker for post-traumatic coagulopathy. Prospects for further research include continu-ing to study the indicators of vascular platelet hemostasis, which, accordingly, may lead to the development of new early diagnostic and therapeutic measures for the prevention and treatment of post-traumatic coagulopathy and its consequences.

CONCLUSIONS

The main pathophysiological changes of vascular-platelet hemostasis in patients with severe trauma appeared in I subgroup on 3rd day, while in II subgroup – on 1st day. For I subgroup the specific changes were decreasing level of discocytes, whereas level of discoechinocytes, spherocytes, spheroechinocytes and sum of active forms of platelets were increased. For II subgroup on 1st day there was increasing sum of active forms of platelets, on 3rd day – level of discocytes was decreased, and levels of discoechinocytes, spherocytes, spheroechinocytes and the sum of active forms of platelets were increased. For both subgroups on 1st and 3rd days bipolar forms of platelets were not observed.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

PHYSICAL THERAPY IN DISABILITY PREVENTION IN LONG-LIVED PERSONS

DOI: 10.36740/WLek202307102

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ABSTRACT

The aim: To evaluate physical therapy in the prevention of disability in long-lived persons.

Materials and methods: The study was conducted over a period of 4 months in a group of 27 patients treated at the Residential Care Facility in Szydłowiec. Most patients were over the age of 90 years (68%), with a mean age of 88 years. They underwent physical therapy cycles and their functional status was documented. Physical therapy included selected physiotherapy and kinesiotherapy methods adjusted to the physical fitness of each patient. Treatment efficacy was monitored using the standardised ADL and Barthel Index scales, which allowed for functional status assessments.

Results: The study found that physical therapy improved physical fitness in study patients after 4 months. Appropriate physical therapy and rehabilitation conducted in this group of patients helped considerably improve their objective and subjective condition, including their functional status and degree of independence.

Conclusions: 1. Chronic musculoskeletal, cardiovascular, nervous system and other disorders are an important aspect of old age. 2. Appropriate physical therapy and rehabilitation in this group of patients helps considerably improve their objective and subjective condition, including their functional status. 3. Providing appropriate care for the elderly is a challenge for healthcare systems.

KEY WORDS: longevity, clinical and social problems, physical therapy

Wiad Lek. 2023;76(7):1517-1526

INTRODUCTION

People have been living longer, with the average lifespan increasing by almost a half over the past century. The proportion of aging and old individuals in the population has been growing, contributing to an increased interest in the oldest generation, whose economic, health, social and psychological needs have to be satisfied. According to the estimates provided by the World Health Organisation (WHO), the overall worldwide number of old individuals is approximately 1 billion, which is 30% of the world's population. The Polish society is also aging. Statistical forecasts show a probability that in 2030 60% of the population will belong to the productive age category and 40% will be elderly, including a higher number of individuals over the age of 75 years [1-7].

Aging is a natural, irreversible and long-term process. Old age can be divided into the following phases: early old age, late old age and longevity. Aging-related changes occur gradually, but their course, severity and signs vary among old individuals [8, 9].

Overall, aging can be associated with such changes as reduced muscle strength, impaired motor coordination, impaired perception of one's surroundings, reduced ability to focus and remember things. An older person becomes weaker, more prone to accidents and injuries and shows worse spatial orientation. People over the age of 70 years can also have very different approaches to life, ranging from optimism and full acceptance of their age to despondency, depression or apathy [8, 9].

It is possible to plan and initiate therapeutic efforts that would allow the elderly to maintain their physical fitness and independence and, consequently, contribute to a good quality of life.

It should be stressed that for many older people loss of physical strength and reduced motor coordination result in a decreased motivation to engage in physical activity, which is also reduced by chronic disease since many disorders are accompanied by pain. Older people may doubt whether forcing themselves to take up physical activity, which they associate with tiredness and increased discomfort, is even worth it.

The process of societal aging is the most important phenomenon of the 21st century both from the point of view of the economy and the rules of social life. The maximum human lifespan refers to the age of a person

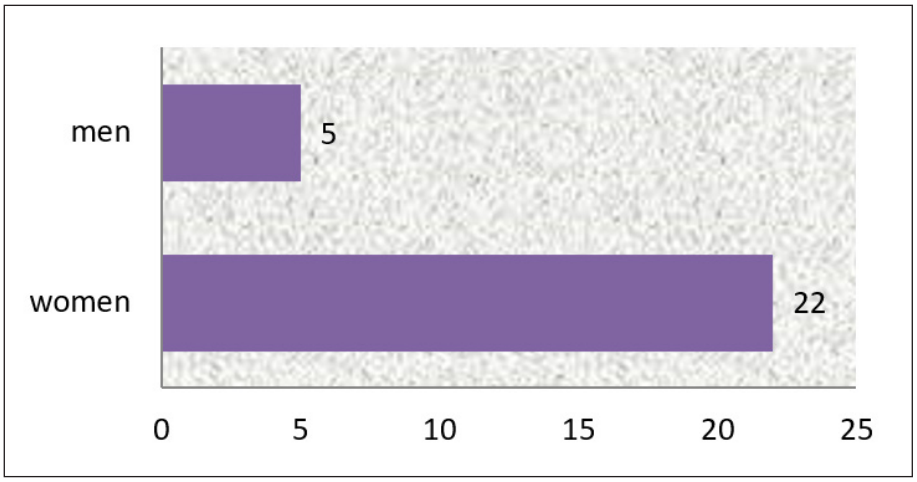

Fig. 1. Sex of study patients

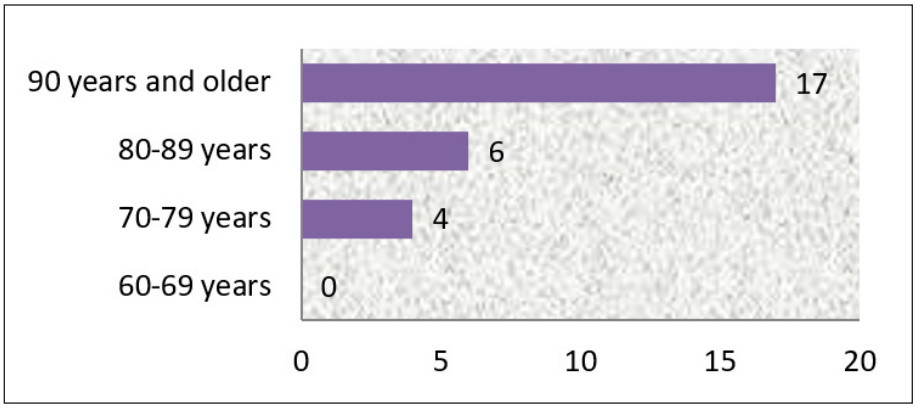

Fig. 2. Age of study patients

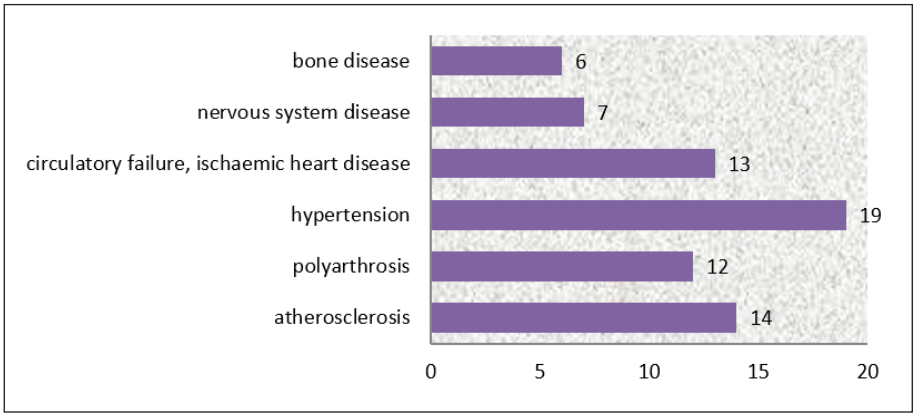

Fig. 3. Morbidity in study patients

and is not subject to typical statistical calculations. There have been individuals who surpassed the age of 110 years. Proven cases of longevity include 122 years in a woman (J. Calment, France, 1875–1997) and 115 in a man (C. Mortensen, Denmark, 1882–1998) [4].

There is no uniform method of dividing old age into periods or uniform criteria that allow for classifying someone as being old in the literature.

The World Health Organisation suggests the following three stages:

- early old age: 60 to 75 years;
- late old age: 75 to 90 years;
- longevity: over 90 years [4,5].

Statistically, over 20% of the population in Poland belongs to the so-called post-productive age category. Of the 6 million people in Poland who are entitled to a retirement pension, more than 1 million individuals are over the age of 80 years.

The issue of motivating elderly people to engage in regular physical activity can be very complicated. Geriatric patients may lack correct habits, be used to a sedentary lifestyle and feel weak. Moreover, they are often reluctant to introduce any changes.

Rehabilitation in the elderly includes kinesiotherapy and physical therapy used to improve fitness and reduce pain. Patients undergo heat therapy, hydrotherapy, laser

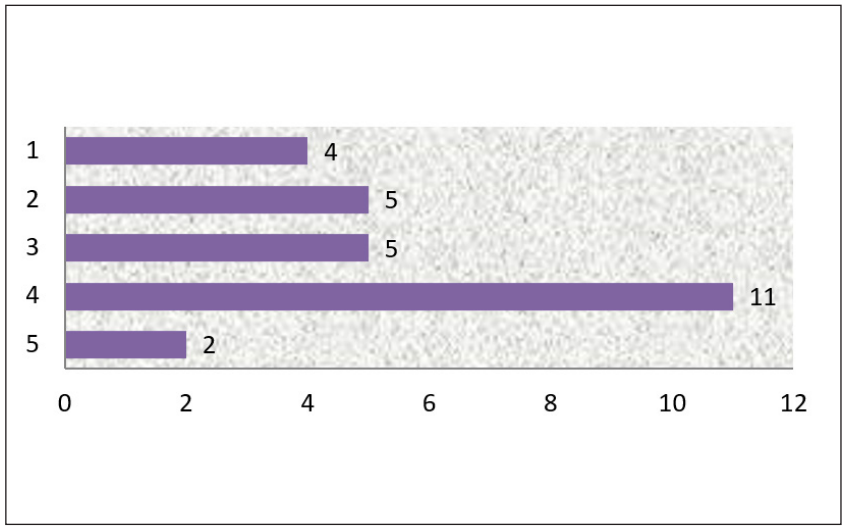

Fig. 4. ADL scores.

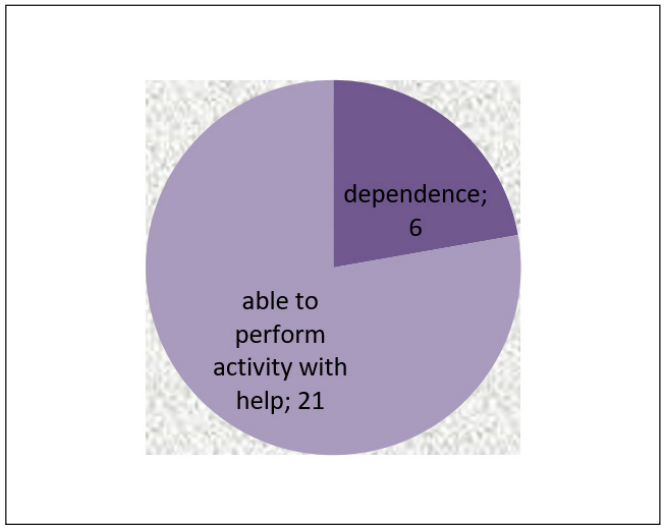

Fig. 5. Degree of impairment according to Barthel Index.

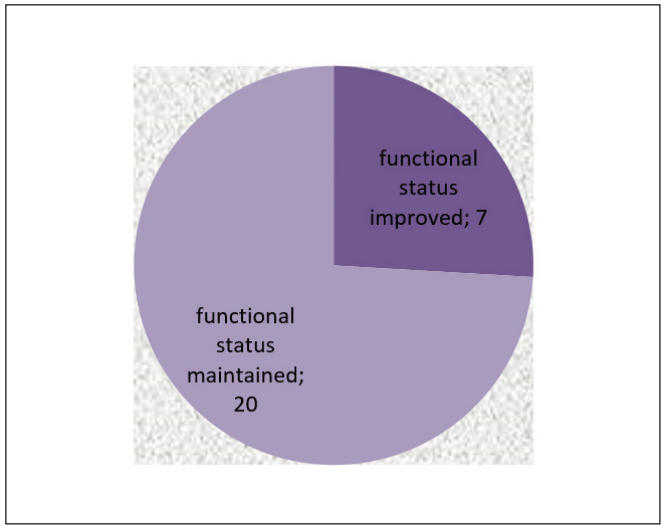

Fig. 6. Functional status improvements according to Barthel Index.

therapy, magnetic field therapy and electrotherapy, but kinesiotherapy is crucial [10-26].

THE AIM

The aim of the study was to evaluate physical therapy in disability prevention in late old age.

What physical therapy methods are effective in the prevention of disability in the elderly?

Detailed hypotheses:

1. The main selection criteria include functional status and needs with respect to rehabilitation; exercise is selected and adjusted to the patient's condition.
2. Treatment efficacy may be monitored using standardised scales, for example the ADL scale or Barthel Index, which helps assess whether the functional status improved or remained unchanged.
3. Patients have a positive opinion about physical therapy.

MATERIALS AND METHODS

The study used the following:

1. method: diagnostic survey;
2. technique: survey, measurement, review of patient records;
3. research tools: survey questionnaire, scales, notes.

Scales used for measurements:

- Katz scale (ADL),
- Barthel Index,
- Shortened Tinetti Test,
- Timed Up and Go Test.

The Katz scale includes six activities of daily living: bathing, dressing, toileting, transferring, feeding and continence. Each activity performed by the patient without assistance means a score of 1 point. The final score is divided as follows:

1. scores of 2 or fewer points: severe functional impairment;
2. scores of 3–4 points: moderate functional impairment;
3. scores of 5–6 points: no impairment [24].

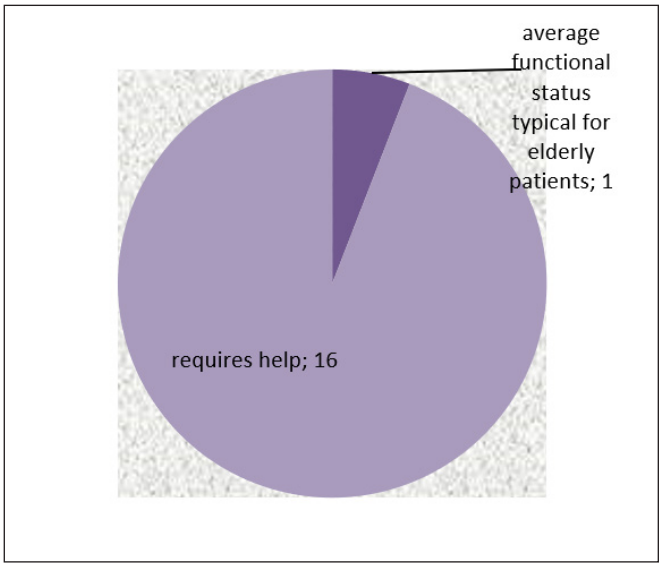

Fig. 7. Functional status according to Timed Up and Go Test

BARTHEL INDEX

The Barthel Index also measures functional status and care requirements. The scale consists of ten activities of daily living, including feeding, transfers, grooming, toilet use, bathing, mobility on level surfaces, using stairs and dressing. The scale assesses the ability to perform these activities unassisted. The final score ranges between 0 and 100 points and can be classified as follows:

- scores of 0 to 20 points indicate total dependency;
- scores of 20 to 80 points indicate that the patient requires help to some extent;
- scores of 80 to 100 indicate that the patient is able to function independently with little help [17].

The Tinetti Test assesses the risk of falls. The balance section of the test measures the following: balance abilities in a sitting position, getting up from a chair, balance immediately after standing up and when standing, being pushed with eyes open and closed, making a 360-degree turn, sitting down.

The gait section of the test encompasses the following: manner of gait initiation, length and height of steps, step symmetry, gait path, trunk movements during gait, feet placement during gait.

The maximum balance score is 16 points and the maximum gait score is 12 points, totalling 28 points. Scores below 26 points indicate a problem and scores lower than 19 points indicate a risk of falls five times higher than that of a person without impairment [17].

The Timed Up and Go Test is a simple test measuring functional status, including mobility as well as static and dynamic balance. The test measures the time an individual needs to get up from a chair, walk a distance of 3 metres, turn around, walk back to the chair and sit down. The results are interpreted as follows:

- up to 10 seconds: normal mobility;

Table I. Correlation between ADL scores and sex and age.

Functional status	Sex		Age		
	female	male	70-79	80-89	90 years and older
Good functional status	2	-	2	-	-
	9.1%	-	50.0%	-	-
Moderate functional status	14	2	2	5	9
	73.6%	40.0%	50.0%	83.3%	52.9%
Total impairment	6	3	-	1	8
	27.3%	60.0%	-	16.7%	47.1%
Total	22	5	4	6	17
Statistic	Chi^2=9.9, p=.018		Chi^2=9.2, p=.024		

Table II. Correlation between Barthel Index scores and sex and age.

Functional status	Sex		Age		
	female	male	70-79	80-89	90 years and older
Able to perform activity with help	18	3	4	5	12
	81.8%	60.0%	100.0%	83.3%	70.6%
Dependence	4	2	-	1	5
	18.2%	40.0%	-	16.7%	29.4 %
Total	22	5	4	6	17
Statistic	Chi^2=8.15, p=.034		Chi^2=7.01, p=.028		

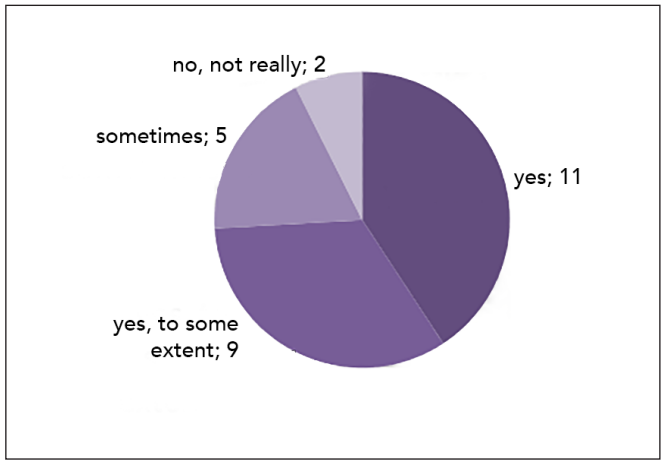

Fig. 8. Receiving information about goal of exercise during rehabilitation.

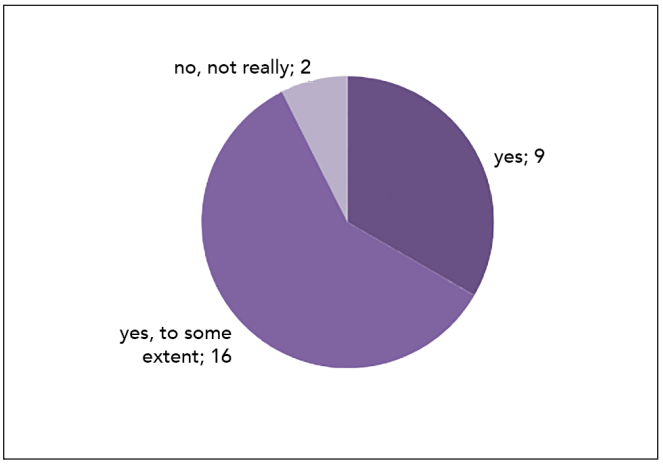

Fig. 9. Willingness to participate in exercise sessions.

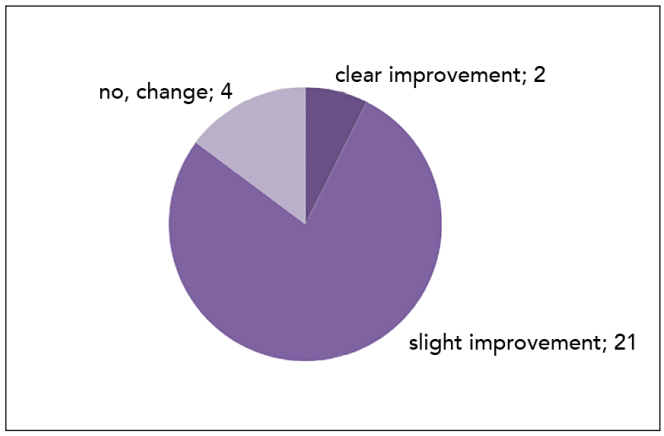

Fig. 10. Influence of exercise sessions on functional status

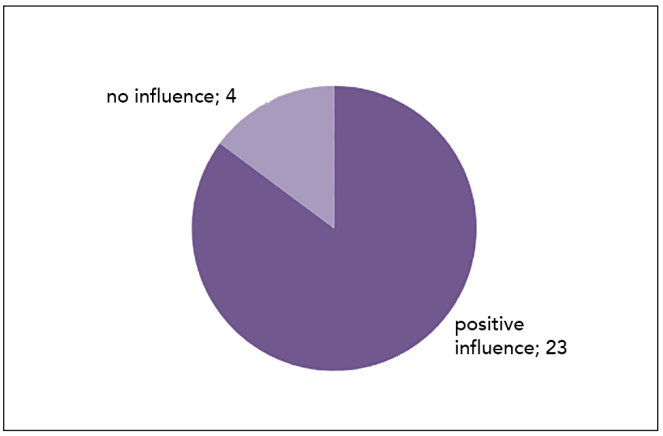

Fig. 11. Influence of exercise sessions on mood.

Table III. Correlation between change in functional status according to Barthel Index and sex and age.

Functional status changes	Sex		Age		
	female	male	70-79	80-89	90 years and older
Functional status maintained	16	4	2	4	14
	72.6%	80.0%	50.0%	66.7%	82.3%
Functional status improved	6	1	2	2	3
	27.3%	20.0%	50.0%	33.3%	17.7 %
Total	22	5	4	6	17
Statistic	Chi^2=12.2, p=.046		Chi^2=8.99, p=.021		

Table IV. Correlation between functional status changes according to Tinetti Test and sex and age.

Functional status changes	Sex		Age		
	female	male	70-79	80-89	90 years and older
Functional status maintained	10	3	1	3	9
	45.5%	60.0%	25.0%	50.0%	52.9%
Functional status improved	12	2	3	3	8
	54.5%	40.0%	75.0%	50.0%	47.1 %
Total	22	5	4	6	17
Statistic	Chi^2=5.49, p=.021		Chi^2=10.17, p=.039		

Table V. Correlation between Timed Up and Go Test results and sex and age.

Functional status	Sex		Age		
	female	male	70-79	80-89	90 years and older
Average functional status typical for elderly patients	1	-	1	-	-
	4.5%	-	25.0%	-	-
Requires help	8	2	3	4	3
	36.4%	40.0%	75.0%	66.7%	17.6 %
High risk of falls	13	3	-	2	14
	40.9%	60.0%	-	33.3%	82.4 %
Total	22	5	4	6	17
Statistic	Chi^2=8.14, p=.037		Chi^2=10.04, p=.019		

- 11–20 seconds: average time for older and disabled individuals;
- more than 20 seconds: patient needs help outside; consider further testing and intervention;
- more than 30 seconds: patient is prone to falls [17].

The scales were applied twice: once at the start of the study and once at the end of the study period. The survey, using a 9-item questionnaire on patient satisfaction, was conducted in the final period of the study.

The results were presented as numbers and proportions. Pearson's non-parametric chi-square test for qualitative data was used to check for statistically significant correlations between the variables (functional status and sex and age). The statistical significance level was set at $p<0.05$. The data were analysed using the StatSoft Statistica 13.1 PL and Microsoft Office software.

The study was conducted in a group of patients treated at the Residential Care Facility in Szydłowiec. The facility is run by the County Office in Szydłowiec.

Study patients were over the age of 75 years. The study group consisted of 27 individuals. The inclusion criteria were as follows:

1. Age: 75 years or older.
2. Patient's consent to participate in the study.
3. Availability: direct patient-investigator contact.

The study was performed over a period of 4 months in 2020 and was approved by the administration of the facility. During the study, patients underwent physical therapy sessions according to an established schedule. The sessions included kinesiotherapy and selected physiotherapy methods. The pre-treatment (baseline) and post-treatment status were recorded individually for each patient so that the combined data could be studied in the analysis of the results.

STUDY GROUP CHARACTERISTICS

The study group consisted of 27 patients, including 22 women and 5 men (Fig. 1).

The majority of study patients were aged 90 years or more (63%) and 6 patients (22%) were aged between 80 and 89 years. The mean age of study patients was 87.6 years (Fig. 2).

Study patients showed multimorbidity. Hypertension was the most common condition (19 patients); other common conditions included atherosclerosis, circulatory failure, ischaemic heart disease, degenerative disease (Fig. 3).

RESULTS

The ADL scores achieved by study patients at the beginning and at the end of the physical therapy period are presented in Figure 4.

The ADL scores in the study patients ranged from 1 to 5 points. The largest group of study patients (11 people) scored 4 points, which indicates moderate impairment. A total of 14 patients scored between 3 and 1 point. The mean score in the study group was 3.07 Correlation between ADL scores and sex and age shows Table I.

Next, the Barthel Index was used for assessments performed at the start and at the end of the physical therapy cycle to evaluate the functional status on the first day of the study and at 4 months (Fig. 4-6,).

The scores obtained in the study group on the first day ranged from 10 to 30 points and the scores calculated at the end of the study were between 10 and 35 points. The mean value for the whole study group was 26.11 at the start of the study and 28.11 at the end.

A statistical analysis was conducted to check for the presence of a correlation between changes in Barthel Index scores occurring in study patients in the physical therapy period and their sex and age (Table II-III). The analysis showed that functional status improvements were achieved in a higher proportion of women as compared to men and were more common in patients aged 75–79 years as compared with older individuals. The statistical analysis confirmed that functional status improvements were easier to achieve when conducting rehabilitation in women, particularly individuals younger than 80 years.

Study patients were also assessed with the Tinetti Test at the beginning and at the end of the study period. It

should be emphasised that all patients achieved low scores in the Tinetti Test, indicating that their risk of falls was several times higher than normal.

The scores were 1–5 points in the initial phase and 2–7 points at the end of the rehabilitation period. Improvements were seen in 14 patients while the functional status of the other 13 individuals remained unchanged.

A statistical analysis was performed to check for the presence of a correlation between functional status changes in the Tinetti Test during physical therapy and sex and age. It showed that there was a correlation between the effects of physical therapy and the sex and age of study patients. Improvements were more often seen in women and patients under the age of 80 years (Table IV).

The Timed Up and Go Test was the last scale used to assess the condition of study patients (Table V, Fig. 7).

The time necessary to perform the activity tested was 19 to 42 seconds at the start of the rehabilitation period and 18 to 41 seconds at the end of rehabilitation. All study patients showed slight functional status improvements after rehabilitation (1 to 3 seconds).

Results of functional status assessments according to the Timed Up and Go Test were statistically analysed in terms of their correlation with sex and age. The proportion of individuals with a high risk of falls was higher in men and in those aged 90 years and older. The statistical analysis confirmed that the correlation between the functional status and the sex and age of study patients was statistically significant (Table V).

Once the study period ended, patients were invited to complete a short survey. The survey asked whether study patients were told about the goal of the exercise they performed during therapy (Fig. 8). Most patients (16 people) were told about it to some extent, 9 patients were definitely told about it and 2 patients were not really told about it.

A total of 17 study patients were given rather clear explanations during rehabilitation and 10 patients reported receiving explanations that were definitely clear.

According to 11 study patients, all types of exercise were difficult to perform whereas for 9 patients difficulties appeared mostly during active exercise. Moreover, difficulties were reported during gait training in 4 patients and during verticalisation in 3 patients.

Most study patients (21 people) did not experience pain during exercise, 4 patients experienced pain rarely and 2 patients reported experiencing pain during exercise.

When asked about their willingness to participate in the exercise sessions, a total of 11 study patients were definitely willing to participate, 9 patients were willing to participate to some extent, 5 patients were willing to participate sometimes and 2 patients were not really willing to perform the exercise (Fig. 9).

According to most study patients (14 people), the duration of the exercise sessions was appropriate, with 7 patients indicating they would be able to exercise longer. According to 2 study patients, the exercise sessions were too long and 4 patients did not have an opinion.

The majority of study patients (21 people) believed their functional status had improved thanks to the exercise sessions, 4 patients stated they saw no change and 2 patients reported a very clear improvement (Fig. 10). No patients reported any negative influence of the exercise sessions on their functional status.

Most study patients (17 people) experienced pain reduction thanks to the exercise sessions.

The majority (23 people) believed the exercise sessions had a positive influence on their mood and 4 patients said the exercise sessions did not have any influence on their wellbeing (Fig. 11). No patients reported any negative influence of the exercise sessions on their mood.

VERIFICATION OF HYPOTHESES

Hypothesis 1: The main exercise selection criteria include functional status and needs with respect to rehabilitation; exercise is selected and adjusted to the patient's condition.

As shown by the study, the functional status is considerably lower in the elderly, mostly with a limited level of mobility and a high risk of falls. Disability prevention should include various types of exercise (passive and active). Most patients accepted the duration of exercise sessions and were told what the goal of the exercise was and how to perform it. However, due to their functional status, many types of exercise are difficult for the patients, with some causing pain, which required programme adjustments. It should be stressed, however, that most study patients achieved a considerable functional status improvement over the 4-month physical therapy period.

The first hypothesis is deemed to be confirmed.

Hypothesis 2: Treatment efficacy may be monitored using standardised scales, for example the ADL scale or Barthel Index, which helps assess whether the functional status improved or remained unchanged.

Four scales were used to monitor the efficacy of therapy. The Katz (ADL) scale is the least sensitive because it is a 6-point tool, which means it has low sensitivity and can only show a limited degree of worsening or improvement. The Barthel Index and the Tinetti Test are more comprehensive and the results of the Timed Up and Go Test are provided in seconds, which makes them easy to analyse.

The second hypothesis is deemed to be confirmed.

Hypothesis 3: Patients have a positive opinion about physical therapy.

Most study patients believed physical therapy had a positive effect on their functional status. According to the vast majority of study patients, participation in exercise sessions had a positive influence on their mood; no patients reported decreased wellbeing as a result of the participation in the exercise sessions. Moreover, most patients were willing to participate in the exercise sessions.

The third hypothesis is deemed to be confirmed.

DISCUSSION

Rehabilitation in the elderly is a social problem and providing care and appropriate rehabilitation for the elderly is a challenge that healthcare systems face today and will continue to face tomorrow. A high proportion of the elderly experience pathological old age with limited physical function. Physical activity may contribute to reducing negative effects of chronic disease [10-16].

According to Łukasik et al. [12], a rehabilitation programme based on resistance exercise allowed for achieving good results in a group of elderly patients with functional limitations due to multimorbidity. The programme improved the condition of muscle by increasing muscle tone and strength and helped compensate for coordination deficits. Overall, it is important for the elderly to not get used to a sedentary lifestyle because it increases the risk of loss of independence. Exercise and rehabilitation in elderly patients improve their self-confidence and encourages them to be active. Rehabilitation must take into consideration any risk factors in order to offer a well-tailored exercise programme with appropriately selected session duration.

The study also showed that planning an exercise session with a patient is a difficult task. The majority of study patients were in the late old age period (63% of patients were aged >90 years; mean age was 88 years) and had multiple comorbidities. The most appropriate type of exercise had to be selected for each case, for example for a female patient with hypertension, diabetes and polyarthrosis. However, the study showed that most patients were willing to participate in rehabilitation and believed that the duration of exercise sessions was well-adjusted to their functional status.

It should also be stressed that exercise that improves or at least maintains the functional status of an elderly patient serves a protective role and is a method of prevention of gait impairment and falls. It is difficult to improve the physical fitness of an elderly person due to physiological changes occurring in the body and pathological changes resulting from disease. However, according to Żak [18], even the most physically impaired people can benefit from motor rehabilitation if the exercise programme is adjusted to their needs and there is no upper age limit at which exercise should be discontinued.

Jajor et al. [19] studied a group of elderly patients and reported that it was difficult to differentiate between age-related health limitations and disease; however, there was definitely a need for motor and functional improvements. Reduced mobility is associated with a gradual decrease in independence because patients worry about the risk of falls and make unsure movements, which further reduces their physical activity. Optimum motor rehabilitation for the elderly includes long-term, systematic training initiated as soon as possible. It is necessary to adjust the exercise to each patient; a person who used to lead a sedentary lifestyle or is undergoing rehabilitation after a period of bed rest should start with short exercise sessions with a gradually increasing duration and load. The authors mostly recommended exercise, elements of yoga and dance and a combination of endurance, strength and resistance training. They pointed out that patients with elevated blood pressure should not undergo isometric exercise, exercise with an excessive number of complex movements and exercise with a large range of flexion and extension.

Rehabilitation was also studied in centres specialising in elderly and disabled persons care [8-10]. The research showed that signs of biological aging are usually evident in gait patterns. It is important to remember that elderly patients learn slowly and increasing their functional status may take over several months. Appropriate care, ensuring a correct position and avoiding joint contractures and muscle atrophy are the most important aspects of the initial phase of rehabilitation. The physiotherapist should ensure appropriate lung ventilation and prevent bedsores. Next, the efforts should focus on increasing the range of motion in the joints and muscle strength. Active-passive exercise, massage, gradual verticalisation and mobilisation of patients to perform simple daily activities without assistance are recommended. Respiratory, antithrombotic and manual exercise requires particular attention while exercise performed solely in a standing position and exercise with trunk twists should be avoided [18-20].

CONCLUSIONS

1. Chronic musculoskeletal, cardiovascular, nervous system and other disorders are an important aspect of old age.
2. Appropriate physical therapy and rehabilitation in this group of patients helps considerably improve their objective and subjective condition, including their functional status.
3. Providing appropriate care for the elderly is a challenge for healthcare systems.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

URINARY TRACT INFECTIONS IN PREGNANT WOMEN IN UKRAINE: RESULTS OF A MULTICENTER STUDY (2020-2022)

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ABSTRACT

The aim: To obtain the first national estimates of the current prevalence rate of urinary tract infections (UTIs) in pregnant women and antimicrobial resistance of causing pathogens in Ukraine.

Materials and methods: Prospective multicentre cohort study was conducted from January 2020 to December 2022. The study population consisted of 36,876 pregnant women from 17 regions of Ukraine. Antibiotic susceptibility was done by the disc diffusion test as recommended by European Committee on Antimicrobial Susceptibility Testing guidelines.

Results: A total 29.5% pregnant women were found to have UTIs. Among these cases, 36.5% Asymptomatic bacteriuria, 51.7% Cystitis and 11.8% Pyelonephritis were observed. Of all cases, 87.9% were defined as healthcare-acquired UTIs and 12.1% community-acquired UTIs. The most common uropathogen was Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa. Many uropathogens isolated from UTI cases were found to be multidrug resistant.

Conclusions: UTIs in pregnant women in Ukraine is a common occurrence and many cases are caused by pathogens that are resistant to antibiotics. Optimizing the management and empirical antimicrobial therapy may reduce the burden of UTIs in pregnant women, but prevention is the key element.

KEY WORDS: pregnant women, healthcare-associated urinary tract infection, community-acquired urinary tract infection, asymptomatic bacteriuria, pyelonephritis, antimicrobial resistance, Ukraine

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INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common microbial diseases among the pregnant women threat worldwide [1]. UTIs are the most common type of infection during pregnancy, affecting from 10% [2, 3] to 24.3% [1] of pregnant women. The spectrum of UTIs in pregnant women ranges from lower urinary tract disease (asymptomatic bacteriuria, acute cystitis) to upper urinary tract disease (acute pyelonephritis). According to literature, in pregnant women the incidence of Asymptomatic Bacteriuria (ASB) in pregnant women were from 2-13% to 57.5% and acute cystitis from 1-4% to 35% [1, 4]. It is reported that acute pyelonephritis may also occur in 1-4% of pregnant women [5]. In Ukraine, the prevalence of pyelonephritis in pregnant women is 7.6% [1].

According to literature, UTIs in pregnant women can lead to complications in maternal and fetal complications such as preterm labor, low birth weight, or maternal systemic infection. It is estimated that every tenth pregnant women with pyelonephritis will eventually go into preterm labor [6]. Untreated UTIs in pregnant women may increase the risk of fetal developmental alterations and mental retardation [7]. In addition, UTIs is closely associated with a higher risk of premature rupture of the membranes, premature birth and early neonatal systemic infection [4, 8]. Therefore, such infections pose considerable diagnostic and therapeutic challenges for pregnant women.

Clinical and therapeutic decisions are influenced by numerous factors, including antimicrobial resistance of the causative agents of UTIs. Current guidelines for man-

agement of UTIs in pregant women recommend the use of antibiotics for treatment infections. However, the growing antimicrobial resistance is limiting their use for treatment of UTIs in pregnant women in Ukraine. There is currently sparse data characterizing antibiotic resistance of common antepartum infections, such as UTIs, and subsequent clinical ramifications. To date, there is limited data describing the incidence and impact of antibiotic-resistance in obstetric infections other than group B streptococcus [9, 10]. However, the potential implications of antibiotic-resistant urinary tract infections are significant [1, 11]. The consequences of antibiotic-resistant infections in non-pregnant patients are better documented than those in pregnancy. For example, antibiotic-resistant blood stream infections lead to both excess mortality and prolonged hospital stays outside of pregnancy [12]. Similar to other infections outside of pregnancy, urinary tract infections in pregnancy are subject to significant antibiotic resistance. Antibiotic stewardship, as well as knowledge of local resistance patterns and appropriate treatment in pregnancy, are critical for improving outcomes and preventing development of worsening resistance patterns. However, prevalence of UTIs in pregnant women and antimicrobial resistance of causing pathogens in Ukraine are scant. The previous reports of UTIs in Ukraine have been limited only to Healthcare-Associated Infections (HAIs) [13, 14].

THE AIM

The aim of this study was to obtain the first national estimates of the current prevalence rate of UTIs in pregnant women and antimicrobial resistance of causing pathogens in Ukraine.

MATERIALS AND METHODS

STUDY DESIGN, SETTINGS AND PARTICIPANTS

We performed a prospective multicenter cohort study was based on surveillance data for UTIs in pregnant women done in 18 women hospitals from 17 Ukrainian regions over 36 months period from January, 2020 to December, 2022. All participants in our study were local residents. The selection criterion for the inclusion in the study was above 18 years. No past history related to any sexually transmitted diseases and immunocompromised status was noted.

DEFINITION

The definition of UTI is an infection in any part of the urinary system, including kidney, ureter, bladder, or urethrae. Urinary tract infection is the presence of the microorganism in the urine. Infections pregnant women were defined as community-acquired urinary tract infection (CA UTI) and healthcare-acquired urinary tract infection (HA UTI). Definitions of healthcare associated urinary tract infection (HA UTI) in pregnant women were used from the CDC/ NHSN guidelines published in 2019. UTIs are classified either as Asymptomatic Bacteriuria (ASB), when the infection is limited to bacterial growth in urine, or symptomatic infections (acute cystitis, acute pyelonephritis), when bacteria invade urinary tract tissues, inducing an inflammatory response. All cases of UTIs was evidenced by urine culture and sensitivity done in urine sample. Two consecutive voided urine specimens (preferably within 2 wk) with the same bacterial species isolated in quantitative count of $>10^5$ CFU/ml in pregnant women were considered to be positive for UTI.

MICROBIOLOGICAL METHODS

We analyzed urine samples from pregnant women's in the context of a study about microbiology of UTIs and antimicrobial resistance of responsible pathogens. Microbial isolates were identified using standard microbiological techniques. Urine samples were obtained from pregnant women with clinical symptoms of UTIs. Urine of all patients was sampled and subjected to routine and microscopy examination and culture. Urine samples were processed by a semi-quantitative dilution method. The significant bacteriuria was 10^5 cfu/ml was taken into consideration while confirmation as UTI. Detected pathogens in significant amounts were identified according to phenotypical characteristics. Antimicrobial susceptibility testing was performed according to EUCAST guidelines at the local laboratories. Isolates with intermediate susceptibility were considered resistant.

DATA COLLECTION

A standard data collection form was created to extract demographic and clinical data, microbiology (isolated pathogens and their antibiograms) and outcome information from routine patient records. Full text relevant hospital records were reviewed for the all pregnant women's. The pregnant women were compared in terms of irritative urinary symptoms, bacteriuria, hematuria, length of hospital stay, and mobilization time. All data were collected using the sign of UTI in this form. In addition, hospital records were scrutinized for the signs and symptoms as per CDC/NHSN criteria for confirmation as HA UTI. Only inpatient samples were considered for analysis. Duplicates were excluded, allowing only

one isolate of a given pathogen per patient. Prevalence of major uropathogenic organisms and their antimicrobial susceptibility patterns were analysed.

ETHICS

Institutional Ethical Committee (IEC) of the Shupyk National Healthcare University of Ukraine (Kyiv, Ukraine) clearance was obtained before beginning of the study. Pregnant women's in the hospital, who accepted to be a part of the study, were approached, IEC clearance certificate was shown to the patients and their consent was obtained. All pregnant women's data were anonymised prior to the analysis.

STATISTICAL ANALYSIS

After all the data was collected, a descriptive analysis was conducted to determine the characteristics of the research subjects. The prevalence of UTIs was reported as the percentage of the total number of pregnant women's. Cases of UTIs were analysed by type of infection (asymptomatic bacteriuria, cystitis and pyelonephritis), which were mutually exclusive. The analysis of statistical data was performed using Excel and SPSS 10.0 statistical software package. Results are expressed as median (range), mean standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. Comparisons were undertaken using Student's t-test and Fisher's exact test for categorical variables. In our study statistical significance was defined as $P<0.05$.

RESULTS

PREVALENCE OF UTIS

During the study period, 10,879 of 36,876 pregnant women were found to have UTIs. The prevalence of UTIs in Ukraine was 29.5% [95% confidence interval (CI) 29.3-29.7%, $P<0.0001$]. The most frequently reported UTI types were: 36.5% (3,970/10,879) asymptomatic bacteriuria, 51,7% (5,625/10,879) cystitis, and 11.8% (1,284/10,879) pyelonephritis. Among these cases, 87.9% (9,563/10,879) were defined as healthcare-acquired urinary tract infection (HA UTI) and 12.1% (1,316/10,879) community-acquired urinary tract infection (CA UTI). The UTIs cases among pregnant women in the participating hospitals varied significantly. In terms of Ukrainian regions, fluctuations of the indicator values were observed of HA UTI in pregnant women – from the smallest in the west and north while higher percentages were reported in the south, east and central region of Ukraine.

CAUSATIVE AGENTS OF UTIS.

In total, 15,281 specimens were isolated from 10,879 pregnant women with UTIs. Of all UTIs 73.1% (7,956/10,879) were reported to be polymicrobial. Overall, Gram-negative bacteria predominated. Considering all UTI types together, *Escherichia coli* (*E coli*) were most commonly reported, accounting for 54.2% of all organisms, followed by *Klebsiella pneumoniae* (14.2% of all organisms), *Proteus mirabilis* (7% of all organisms), and *Pseudomonas aeruginosa* (5.5% of all organisms). These were the same organisms reported most commonly for HA UTI cases (Table I).

ANTIMICROBIAL RESISTANCE OF RESPONSIBLE PATHOGENS

Antimicrobial susceptibility tests were performed on a total of 1694 isolates of Gram-positive cocci and 13525 isolates of Gram-negative bacilli. The staphylococcal isolates (*S.aureus*, CoNS) displayed a high resistance to penicillin (73.5%) and erythromycin (65.7%), although there were some differences depending on the species. In this study staphylococcal isolates showed susceptibility to most of the other antimicrobials tested. Among staphylococcal isolates no strains resistant to linezolid, teicoplanin, vancomycin, tigecycline, and fusidic acid were found. Methicillin-resistance was observed in 8.1% of *S. aureus* (MRSA) and 11.6% CoNS. Streptococcal isolates demonstrated a high resistance against erythromycin (55.6%) and benzylpenicillin (64.8%), followed by ampicillin (31.7%) and tigecycline (16.7%). Most of streptococcal isolates were sensitive to rifampicin (86.3%), clindamycin (89.9%), gentamycin (94.1%), cefuroxime (95.2%), tobramycin (98.9%), and linezolid (99.8%). Regarding the genus *Enterococcus*, *E. faecalis* isolates were not sensitive to those antibiotics to which they are intrinsically resistant (cefuroxime, clindamycin, and trimethoprim-sulfamethoxazole) and 72.8% of them were resistant to erythromycin. Approximately, 20% of the *E. faecalis* isolates displayed resistance to high levels of aminoglycosides (gentamycin, tobramycin) and around 11.9% was resistant to quinolones (ciprofloxacin and levofloxacin), and 4.3% to glycopeptides (vancomycin and teicoplanin). Vancomycin resistance was observed in 5.2% of isolated enterococci (VRE). *Enterobacter* spp. was most sensitive (>90%) to ciprofloxacin (97.1%), piperacillin/tazobactam (95.8%), ceftriaxone (93.2%), ceftazidime (93.1%), and fosfomycin (92.1%). No strains resistant to cefepime, meropenem, imipenem, and ertapenem were found. In the present study the most resistant uropathogens were *E. coli, K. pneumoniae, P. mirabilis*

and *P. aeruginosa* which showed the high resistance to multiple antibiotics. The overall proportion of extended spectrum beta-lactamases (ESBL) production among *Enterobacteriales* was 25.7%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (33.7%, vs 14.8%, $p < 0.001$). Resistance to third generation cephalosporins was observed in 13.9% *E.coli* isolates and in 10.1% *K. pneumoniae* isolates, respectively. Carbapenem resistance was identified in 13.7% of *P.aeruginosa* isolates. Antibiotic resistance patterns of the most frequent causative uropathogens in pregnant women in Ukraine are presented in Table II.

There was no significant difference in the resistance profile of *E. coli*, *K. pneumoniae, P. mirabilis*, and *P. aeruginosa* across regions or hospital category. Overall, 24.5% of all HA UTI samples were obtained from primary care centers, 35.8% were obtained from secondary care centers, and 39.7% were obtained from tertiary medical centers. The distributions of pathogens were similar within the 3 types of centers (Primary, Secondary and Tertiary). There was no significant difference in resistance profiles from uropathogens between different types of centers (Table III).

DISCUSSION

In the present study, we report the prevalence of UTIs in pregnant women and antimicrobial resistance rates for major causative agents in Ukraine. Although many studies have already described increasing resistance rates in urinary isolates, Ukrainian data are limited either to single centre studies or to studies focusing on resistance to single antibiotic classes. The prevalence of UTIs in pregnant women was 29.5%. Among these patients, 36.5% ASB, 51.7% Cystitis and 11.8% Pyelonephritis were observed. Of all UTI cases 87.9% were defined as HA UTI and 12.1% CA UTI. The prevalence of UTIs in this study was relatively higher compared to findings in other published studies. Other studies report an UTI incidence rate of 10% among pregnant women [2, 3]. It is estimated in pregnant women the incidence of ASB in pregnant women were 2-13% [4,15] and acute cystitis in 1-4% [4,16]. It is reported that acute pyelonephritis may also occur in 0.5-4% of pregnant women [5,17]. Thus, the UTI in pregnant women in Ukraine is much higher than in other countries. In this study, UTIs were polymicrobial. The predominant pathogens were *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*. Our finding was in line with other studies [1, 18, 19]. Our

Table I. Microorganisms causing of Urinary Tract Infections (UTIs) in pregnant women in Ukraine (2020-2022)

Microorganisms	All isolates No. (%)	HA UTI No. (%)	CA UTI No. (%)
Gram-positive cocci	1694 (11.1)	926 (54.7)	768 (45.3)
Enterococcus faecalis	676 (4.4)	397 (58.7)	279 (41.3)
Enterococcus faecium	65 (0.4)	38 (58.5)	27 (41.5)
Streptococcus spp.	306 (2.0)	168 (54.9)	138 (45.1)
CoNS	524 (3.4)	236 (45.0)	288 (55.0)
Staphylococcus aureus	123 (0.8)	87 (70.7)	36 (29.3)
Gram-negative bacilli	13525 (88.5)	11031 (81.6)	2494 (18.4)
Escherichia coli	8280 (54.2)	7369 (89.0)	911 (11.0)
Klebsiella pneumoniae	2166 (14.2)	1708 (78.9)	458 (21.1)
Klebsiella oxytoca	185 (1.2)	88 (47.6)	97 (52.4)
Enterobacter spp.	424 (2.3)	188 (44.3)	236 (55.7)
Proteus mirabilis	1068 (7.0)	699 (65.4)	369 (34.6)
Serratia spp.	189 (1.2)	121 (64.0)	68 (36.0)
Citrobacter spp.	226 (1.5)	133 (58.8)	93 (41.2)
Pseudomonas aeruginosa	847 (5.5)	628 (74.1)	219 (25.9)
Acinetobacter spp.	140 (0.9)	97 (69.3)	43 (30.7)
Fungi	62 (0.4)	29 (46.8)	33 (53.2)
Candida albicans	62 (0.4)	29 (46.8)	33 (53.2)
Total	15281 (100.0)	11986 (78.3)	3295 (21.7)

CoNS, Coagulase-negative staphylococci;
CA UTI, community-acquired urinary tract infection;
HA UTI, healthcare-acquired urinary tract infection

Table II. Resistance pattern of the main causative agents of UTIs in pregnant women in Ukraine (2020-2022)

Pathogen	Antibiotic	HA UTI n/R (%)	CA UTI n/R (%)	P value
Escherichia coli	CRO	7369/857 (11.6)	911/75 (8.2)	<0.001
	CIP	7288/1897 (26.0)	906/153 (16.9)	<0.001
	NOR	7369/2071 (28.1)	877/168 (19.1)	<0.001
	TMP-SMX	2364/648 (27.4)	893/155 (17.4)	<0.001
	FFM	1987/45 (2.3)	902/13 (1.4)	0.018
	AMC	1403/408 (29.1)	897/215 (24.0)	<0.001
	NIT	1276/35 (2.7)	905/15 (1.6)	0.018
Klebsiella pneumoniae	CRO	1708/173 (10.1)	458/29 (6.3)	0.041
	CIP	1627/290 (17.8)	417/48 (11.4)	0.001
	NOR	1588/261 (16.4)	455/54 (11.8)	0.031
	TMP-SMX	1687/221 (13.1)	433/55 (12.7)	0.927
	FFM	1702/488 (28.7)	427/131 (30.7)	0.479
	AMC	1691/301 (17.8)	311/40 (13.0)	0.0412
	NIT	305/206 (67.5)	417/279 (66.9)	0.837
Proteus mirabilis	CRO	588/15 (2.5)	319/6 (1.8)	0.437
	CIP	693/149 (21.5)	347/53 (15.3)	0.05
	NOR	678/147 (21.7)	358/47 (13.1)	0.012
	TMP-SMX	683/209 (30.6)	311/98 (31.5)	0.819
	FFM	654/151 (23.1)	357/62 (17.4)	0.05
	AMC	699/89 (12.7)	369/24 (6.5)	0.012
	NIT	576/576 (100.0)	209/205 (98.8)	0.365
Pseudomonas aeruginosa	CIP	628/92 (14.6)	219/24 (10.9)	0.115
	CEF	581/80 (13.7)	215/17 (7.9)	0.013
	PIP	621/91 (14.6)	207/20 (9.7)	0.041
	CAZ	607/65 (10.7)	211/17 (8.1)	0.252
	IMI	595/82 (13.8)	212/22 (10.4)	0.212

CRO, Ceftriaxone; CIP, Ciprofloxacin; NOR, Norfloxacin; TMP-SMZ, trimethoprim-sulfamethoxazole; FFM, fosfomycin; NIT, Nitrofurantoin; AMC, Amoxicillin/clavulanic acid; CEF, cefepime; PIP, piperacillin-tazobactam; CAZ, ceftazidime; IMI, Imipenem

study showed that UTI in pregnant women in Ukraine were significantly associated with pathogens resistant to antibiotics. The overall proportion of extended spectrum beta-lactamases (ESBL) production among Enterobacteriaceae was 25.7%. The prevalence of ESBL production among E. coli isolates was significantly higher than in *K.pneumoniae*. Resistance to third-generation cephalosporins was observed in 10.1% *K. pneumoniae* and *E. coli* 13.9% isolates. MRSA was observed in 8.1% of *S.aureus* and 11.6% CoNS. VRE was observed in 5.2% of isolated enterococci. Carbapenem resistance was identified in 13.7% of *P.aeruginosa* isolates. Possibly, higher incidence rate of UTIs in pregnant women in Ukraine were significantly associated with antimicrobial resistance of responsible pathogens. In this study the uropathogens isolated from HA UTI cases were found to be multidrug resistant. These findings correlate with various other studies [1, 11, 15, 18, 19] where multidrug resistant uropathogens were isolated.

The resistance rates of Gram-negative uropathogens to a majority of commonly used antimicrobials in the present study were high, a fact to be expected given the epidemiological situation in the world today. With increasing individual mobility and international travel easier than ever, a global spread of multi-drug-resistant bacterial strains seems an inevitable reality. Increase in the antibiotic resistance amongst the uropathogens indicates that they are hospital acquired and thus difficult to treat. This will be more dangerous if infection prevention practices are not followed during care of the catheterized patients. The chances of transmission of these multi drug resistant are high if health care workers do not follow preventive practices meticulously. In the present study the incidence is much lower because of

Table III. Resistance profile of the main causative agents of HA UTIs in pregnant women in Ukrainian hospitals (2020-2022)

Pathogen	Antibiotic	Hospital category			P value
		Primary n/R (%)	Secondary n/R (%)	Tertiary n/R (%)	
Escherichia coli	CRO	707/78 (11.1)	374/34 (9.1)	233/40 (17.2)	0.0081
	CIP	646/159 (24.7)	333/81 (24.3)	203/66 (32.5)	0.0637
	NOR	711/205 (28.9)	303/83 (27.4)	237/63 (26.6)	0.749
	TMP-SMX	714/183 (25.4)	405/108 (26.7)	306/101 (33.0)	0.0411
	FFM	685/16 (2.3)	412/9 (2.2)	308/7 (2.3)	0.975
	NIT	679/14 (2.1)	392/4 (1.0)	205/7 (3.4)	0.128
	AMC	707/166 (23.5)	384/121 (31.5)	312/121 (38.8)	<0.001
Klebsiella pneumoniae	CRO	213/16 (7.5)	139/10 (7.2)	84/13 (15.5)	0.0642
	CIP	187/29 (15.5)	113/17 (15.0)	70/20 (28.6)	0.0312
	NOR	208/37 (17.8)	100/15 (15.0)	91/12 (13.2)	0.565
	TMP-SMX	199/20 (10.1)	137/17 (12.4)	115/22 (19.1)	0.0631
	FFM	153/30 (19.6)	113/45 (39.8)	75/23 (30.7)	0.001
	NIT	164/115 (70.1)	85/54 (63.5)	56/37 (66.1)	0.547
	AMC	200/29 (14.5)	138/23 (16.7)	120/23 (19.2}	0.535
Proteus mirabilis	CRO	96/1 (1.0)	65/0 (0.0)	47/4 (8.5)	0.0061
	CIP	84/21 (25.0)	49/8 (16.3)	39/8 (20.5)	0.487
	NOR	99/25 (25.3)	44/7 (15.9)	43/8 (18.6)	0.378
	TMP-SMX	97/27 (27.8)	63/18 (28.6)	59/22 (37.3)	0.419
	FFM	70/20 (28.6)	46/9 (19.6)	38/5 (13.2)	0.154
	NIT	98/98 (100.0)	38/38 (100.0)	23/23 (100.0)	-
	AMC	95/7 (7.4)	61/3 (4.9)	60/5 (8.3)	0.654
Pseudomonas aeruginosa	CEF	142/21 (14.8)	100/17 (17.0)	87/7 (8.0)	0.173
	CIP	148/26 (17.6)	123/17 (13.8)	79/8 (10.1)	0.311
	PIP	126/21 (16.7)	130/20 (15.4)	88/9 (10.2)	0.378
	CAZ	152/18 (11.8)	129/14 (10.9)	88/7 (8.0)	0.614
	IMI	139/20 (14.4)	90/13 (14.4)	84/10 (11.9)	0.745

CRO, Ceftriaxone; CIP, Ciprofloxacin; NOR, Norfloxacin; TMP-SMZ, trimethoprim-sulfamethoxazole; FFM, fosfomycin; NIT, Nitrofurantoin; AMC, Amoxicillin/clavulanic acid; CEF, cefepime; PIP, piperacillin-tazobactam; CAZ, ceftazidime; IMI, Imipenem

continuous monitoring and training of the staff. This is achieved because of the active Infection control team and surveillance for non-compliances.

UTIs are mainly caused by Gram-negative bacteria that are becoming an increasing threat to public health because of their ability to acquire genes, located on transferable plasmids, that code for extended-spectrum β-lactamases (ESBLs). These enzymes are capable of hydrolyzing third-generation cephalosporins and monobactams but not carbapenems. In addition, ESBLs pose a public health problem because they are encoded on plasmids that usually carry other resistance genes against different classes of antibiotics (e.g., aminoglycosides, sulfonamides, and quinolones). As a result, bacteria that acquire these plasmids become multidrug resistant. Although all ESBLs function through cleavage of the amide bond of the β-lactam ring, the genes encoding these enzymes are diverse and grouped into different families. CTX-M type enzymes are the most commonly encountered ESBL types, being present in several members of the order *Enterobacterales* in *P. aeruginosa* and *Acinetobacter* spp. Isolated strains carrying CTX-M confer high-level resistance to cefotaxime and have reduced susceptibility to ceftazidime. Other types of ESBLs are OXAs, AmpCs, and Carbapenemases. OXAs and AmpC are β-lactamase enzymes encoded by chromosomal and plasmid genes that resist inhibition by β-lactamase inhibitors. *K. pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1) are enzymes that make Enterobacteriaceae resistant to a wide range of beta-lactam antibiotics, particularly carbapenemases (CRE) [14, 15]. The most prevalent (TEM

+ CTX-M) genes were also detected in ciprofloxacin resistant strains *P. mirabilis* and *E. coli* [20]. Because of high prevalence of MDR strains in epidemiologically unrelated patients with AmpC- and/or ESBL producing Proteus spp. infection, further surveillance is needed [21]. Other important mechanisms of resistance are limitation of absorption of a drug, modification of a drug target, and active efflux of a drug. Some bacterial proteins are targets of antimicrobials. Alteration of these bacterial proteins so that the drug binds poorly or does not bind at all is a common mechanism of resistance [19].

We suggest that increased efforts should focus on the prevention and control of hospital infections, and the management of antimicrobial clinical applications to maximize bacterial resistance surveillance. According to literature, antibiotic consumption is a primary driver for AMR, a fact documented on a hospital, regional, and country level [22]. Therefore, the knowledge of local and regional antimicrobial susceptibility patterns is one of the ways to improve antibiotic prescription and at least in part to counter the development of AMR. Monitoring of pathogen resistance profiles is necessary to guide empirical antibiotic therapy before culture and sensitivity results become available. Despite the existence of international guidelines, research shows improper antibiotic prescribing is commonplace [23]. According to a survey from the United States, 30% of primary care antibiotic prescriptions were classified as inadequate. Chardavoyne et al. reported appropriate antibiotic treatment in 68% of adult cystitis cases and 46% of pyelonephritis cases [24]. Treatment in the absence of infection is common and although not recommended in international guidelines for lower UTI management, fluoroquinolones are frequently prescribed.

Our study motivates several potential future areas of research in Ukraine. It would be interesting to examine patterns of resistance in a multi-center study of bacteriuria in pregnant women. Additionally, this work could be expanded to examine antibiotic resistance patterns in other obstetric infections. Finally, in Ukraine data on carbapenem resistance was not available at the time this study was completed. Carbapenem-resistance is an emerging threat identified by the Centers for Disease Control and Prevention, and further investigation into the prevalence of carbapenem-resistant infections in pregnancy is warranted in future work [25].

STRENGTHS AND LIMITATIONS

The biggest strength of this study was the large data set. We analyzed data from 17 regions covering ~70,8% territory of Ukraine. We believe our findings are a solid representation of the current ecology of urinary pathogens in Ukraine. Our data, together with other papers in the literature, complement the picture of AMR worldwide and its trends. Our study provides important new insights into the implications of antibiotic-resistance in pregnancy, but is subject to several limitations. Present study population had a high rate of progression to pyelonephritis which we attributed partially to the inclusion of only culture proven gram negative infections, however differences in clinical practice may also be contributing factors. Data on urine samples collected as a test of cure for initial infections was difficult to collect in a retrospective fashion due to the variable timing of collection and inconsistent documentation. Therefore, rates of cure were not included in this study. The absence of this data makes it difficult to determine whether repeat positive urine cultures were due to re-infection or persistent, or inadequately treated, infection. This limited our ability to assess implications of antibiotic-resistance on obstetric outcomes, such as preterm birth.

CONCLUSIONS

The present study showed that UTIs in pregnant women in Ukraine is a common occurrence and many cases are caused by pathogens that are resistant to antibiotics. Most UTIs in pregnant women are treated empirically with antibiotics, making comprehensive resistance surveillance data essential to guide empiric regimens. A urine culture before initiating antibiotics is essential and the therapy should subsequently be adapted. Multi-faceted interventions, including reinforcement of hand hygiene and control of antibiotic use, are necessary to prevent the spread of MDR uropathogens. Optimizing the management and empirical antimicrobial therapy may reduce the burden of UTIs in pregnant women, but prevention is the key element.

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ORIGINAL ARTICLE

THE ROLE OF THROMBOSIS RISK SCALES LIKE PROGRESSION PREDICTORS OF COVID-19-ASSOCIATED PNEUMONIA

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ABSTRACT

The aim: To determine the risk factors for progression by establishing the diagnostic and prognostic role of PPS and ISTH DIC score in patients with COVID-19 required hospitalization.

Materials and methods: Main group was 130 patients with COVID-19, divided depending on the severity into 3 subgroups. Patients were examined twice. On visit 1 and visit 2 (after 7–14 days after hospitalization). Were provided: physical examination, lung ultrasound (LUS), laboratory tests (D-dimer, coagulogram). Were counted PPS and ISTH DIC score. Non-parametrical statistic, ROC analysis.

Results: The level of D-dimer was significantly elevated in the examined patients and correlated with the severity of the disease. The number of points on the scales ISTH DIC and Padua had a significant difference between the subgroups. The maximum number of points was obtained by patients of subgroup 3, which indicates the maximum risk of thrombotic complications, as well as DIC. The ROC analysis showed that among coagulation parameters the most sensitive and specific factors associated with the progression of the clinical course is the D-dimer at admission.

Conclusions: Thus, the determination of D-dimer, fibrinogen, as well as usage of the DIC and Padua scales is a useful tool not only to estimate the severity of COVID-19, but also to predict the prognosis. Thus, the level of D-dimer above 260 ng/ml, as well as the presence of three or more points on the DIC scale and/or five or more points on the Padua scale in patients with COVID-19 during hospitalization significantly increases the risk of progression clinical process.

KEY WORDS: coagulation, viral pneumonia, COVID-19, predictors of progression, Padua score, ISTH Criteria for Disseminated Intravascugulation

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INTRODUCTION

Despite a strong fight with coronavirus disease (COVID-19), unfortunately, a lot of them die because of different complications [1]. One of the most dangerous complications of COVID-19 is thrombosis, which is diagnosed approximately in 20% of cases [2, 3]. But much more often we face with pulmonary endo-thrombosis, which is non-massive and progresses gradually [4, 5]. This complication has a subclinical course, when the deterioration occurs slowly: shortness of breath progresses daily, saturation decreases, oxygen therapy becomes ineffective. Diagnosis of pulmonary microthrombosis is extremely difficult for verification and treatment.

Another major clinical manifestation noted in COVID-19 patients is disseminated intravascular coagulation (DIC) [6], which is the result of severe systemic inflammation and thrombotic microangiopathies [7]. It is the most critically complication, which leads to mortality and practically uncorrected.

That is why scientists around the world are searching for clinical and laboratory markers, which could be fast and cheap predictors of COVID-19 complications including thrombosis and disseminated intravascular coagulation (DIC) [3, 8, 9].

Today the most popular way to estimate the risk of pulmonary thromboembolism is to use Padua prediction score (PPS), which is already demonstrated its effective application and described by us in severe bacterial community-acquired pneumonia [6, 10, 11], however, COVID-19 pneumonia, this method needs further study. Another modern option for today is to use Criteria for DIC, which were worked out by International Society on Thrombosis and Haemostasis (ISTH) and became popular in surgery and cardiology [7]. But there are no published works about its role in COVID-19 patients.

THE AIM

Thus, the aim of our study was to determine the risk factors for the pathological process progression by establishing the diagnostic and prognostic significance of PPS and ISTH DIC score in patients with COVID-19 required hospitalization.

MATERIALS AND METHODS

We screened 230 adults (mean age – 51 [33; 66] years, men – 122 (53,0%)) with confirmed by polymerase chain reaction (PCR) COVID-19 together with clinically and radiologically signs of pneumonia. We excluded all patients not required the hospitalization, with clinically significant comorbidity that could affect the results of the study (including HIV, tuberculosis, malignancy, severe cardiovascular, renal and hematological pathology), and also all patients who are constantly receiving basic anticoagulant or anti-inflammatory therapy in connection with concomitant conditions, which could distort the data obtained by us.

Main group was 130 patients with COVID-19, divided depending on the severity into 3 subgroups: subgroup 1 – patients with moderate severity (with the development of pneumonia without respiratory failure), subgroup 2 – patients with severe course (with the development of pneumonia with respiratory failure), subgroup 3 – patients with critical course (complicated with acute respiratory distress syndrome, sepsis or multiorgan failure).

Patients were examined twice. On visit 1 (after hospitalization) we provided: vital signs, physical examination with body mass index (BMI), local laboratory and instrumental (radiograph and/or chest computer tomography (CT)), pulse oximetry, lung ultrasound (LUS). Special laboratory tests included platelet count, D-dimer, prothrombin time, fibrinogen. Venous blood sampling for analysis was performed before the appointment of standard therapy; the results of the indicators were evaluated in comparison with the laboratory reference values [6, 11]. Additionally, we counted PPS [12] and ISTH DIC score [12].

On visit 2 (after 7–14 days after hospitalization) we fixed the results of COVID-19 pneumonia patient treatment as failure (in case of progression of the patient's clinical symptoms (with increased dyspnea and increased respiratory rate (RR) by more than on 5 movements, accompanied by decreasing of blood saturation (SpO_2) by more than on 4% compared to baseline) or successful (in case of stabilization of the disease with gradual improvement of symptoms).

Treatment of patients was carried out according to national recommendations. Patients in subgroup 1 received anticoagulant therapy (subcutaneous administration of enoxaparin or analogues in prophylactic doses), anti-inflammatory drugs (nonsteroidal drugs (aspirin, paracetamol, ibuprofen)), low doses of steroids (dexamethasone, methylprednisolone), antibacterial drugs if needed (amoxicillin/ clavulanate or second or third generation cephalosporins (cefuroxime, ceftriaxone)). Patients in subgroup 2 received anticoagulant therapy (subcutaneous administration of enoxaparin or analogues in high prophylactic doses), anti-inflammatory drugs (nonsteroidal drugs (aspirin, paracetamol, ibuprofen)) and low or medium doses of steroids (dexamethasone, methylprednisolone), antibacterial drugs if needed (protected penicillins (amoxicillin/clavulanate) or cephalosporins of the II or III generation (cefuroxime, ceftriaxone)) with a macrolide (azithromycin, clarithromycin), in case of ineffectiveness – respiratory fluoroquinolones (levofloxacin, moxifloxacin). Patients in subgroup 3 received anticoagulant therapy (subcutaneous administration of enoxaparin or analogues in high prophylactic doses, and in confirmed thrombosis – in therapeutic doses), anti-inflammatory (nonsteroidal (aspirin, paracetamol, ibuprofen)) and medium or high doses of steroids, antibacterial therapy (beta-lactams or respiratory fluoroquinolones), in case of ineffectiveness, reserve drugs (linezolid or meropenem) were added, as well as oxygen therapy.

On visit 2, despite the prescribed treatment according to the severity of the disease, a certain proportion of patients showed progressive deterioration, which was mainly characterized by an increase of respiratory failure: increased shortness of breath, decreased saturation, and increased tachypnea. Thus, only 33 of 82 (40,0%) patients of subgroup 1 did not experience worsening of clinical symptoms, while 49 (60,0%) patients, despite adequate treatment, had an increase RR and decreased saturation to severe (less 92%) or critical (less than 85%) level (in 46 and 3 cases, respectively). In subgroup 2, the progression of respiratory failure to the critical level was observed in 31 of 42 (73,8%) patients; 11 others had a stable course with a gradual improvement in clinical condition. Thus, at visit 2 it was found out that among 124 patients with moderate and severe COVID-19 pneumonia there were 80 cases (64,5%) with progression of the pathological process.

To determine the factors that could be recognized as risk markers for the progression of COVID-19-associated pneumonia, we conducted a ROC analysis. Various clinical and laboratory parameters were selected as variables upon admission to the hospital, and as a classification grouping – the fact of pathology progression.

Based on the obtained data, conclusions were drawn on the diagnostic and prognostic significance of the main clinical and laboratory parameters in patients with COVID-19 of different severity.

The analysis of the normality of the distribution of indicators was performed by the Shapiro-Wilk test, testing the hypothesis of equality of variances was performed according to the Levene's test. Continuous variables are given as the median (Me) and the interquantile range (for non-normally distributed

Table I. Some clinical parameters of patients with COVID-19 pneumonia at admission, Me (25 %;75 %)

Parameters	Main group	Subgroups			p
		1 (n=82)	2 (n=42)	3 (n=6)	
Mean age, years	57,5 (51; 65)	57,0 (51; 65)	60 (52; 69)	46 (45; 56)	p_{1-2}=0,453 p_{1-3}=0,008 p_{2-3}=0,102
Male gender (%)	63 (48,5)	32 (39,0)	27 (67,5)	3 (50,0)	p_{1-2-3}=0,127
Axillary temperature, °C	38,5 (38,1; 38,9)	38,0 (37,9; 38,6)	38,8 (38,7; 39,0)	38,9 (38,5; 40,0)	p_{2-3}=0,007 p_{2-4}=0,017 p_{3-4}=0,700
HR per 1 min	90 (85; 100)	88 (80; 94)	90 (86; 100)	110 (100; 125)	p_{2-3}=0,248 p_{2-4}=0,004 p_{3-4}=0,012
RR per 1 min	20 (18; 21)	18 (16; 21)	20 (20; 21)	24 (23; 27)	p_{2-3}=0,001 p_{2-4}=0,000 p_{3-4}=0,000
SpO_2, %	93 (92; 96)	96 (94; 98)	93 (92; 93)	78 (74; 89)	p_{2-3}=0,000 p_{2-4}=0,000 p_{3-4}=0,001

Notes: p – the significance of the difference between the subgroups by Mann-Whitney; 1, 2, 3, 4 – the corresponding subgroups of patients, HR – hart rate, RR – respiratory rate, SpO_2 – blood saturation

Table II. Some coagulation parameters in patients with COVID-19 at admission, Me (25 %; 75 %)

Parameters	Subgroups			Laboratory references	p
	1 (n=82)	2 (n=42)	3 (n=6)		
D-dimer, ng/ml	290,14 (109,30; 660,46)	1449,68 (269,00; 2480,00)	5550,00 (3200,00; 7500,00)	0–285	p_{2-3}=0,001 p_{2-4}=0,001 p_{3-4}=0,006
PT, sec	13,65 (12,0; 14,8)	16,00 (13,0; 18,0)	15,35 (15,20; 16,00)	11–15	p_{2-3}=0,001 p_{2-4}=0,002 p_{3-4}=0,841
Fibrinogen, g/l	4,00 (3,50 5,60)	4,50 (3,69; 6,00)	7,00 (6,50; 7,50)	2–4	p_{2-3}=0,232 p_{2-4}=0,005 p_{3-4}=0,010
ISTH DIC score	2 (2; 4)	4 (3; 5)	4 (4; 5)		p_{2-3}=0,001 p_{2-4}=0,001 p_{3-4}=0,242
PPS	5 (4; 6)	5 (5; 6)	6 (6; 6)		p_{2-3}=0,015 p_{2-4}=0,021 p_{3-4}=0,045

Notes: p – the significance of the difference between the subgroups by Mann-Whitney; 1, 2, 3, 4 – the corresponding subgroups of patients, PT – prothrombin time, ISTH DIC – Criteria for Disseminated Intravascular Coagulation by International Society on Thrombosis and Haemostasias, PPS – Padua Prediction Score

variables). The quantitative indicators were compared by Mann-Whitney (non-parametric criterion). The categorical variables were compared using the Pearson's chi-squared test ($\chi 2$) (Yates correction for traits less than 10). The receiver operating characteristic (ROC) curves were analyzed to determine the prognostic level of parameters. The relative risk (RR) of re-hospitalization and mortality was calculated with a 95% confidence interval (CI). P-value less than 0.05 were considered as statistically significant. Statistical processing of the obtained results was performed by biometric analysis methods implemented in the software packages «STATISTICA 6.0» (№ 31415926535897) and MedCalc, 18.9.1 (free version) software packages [12].

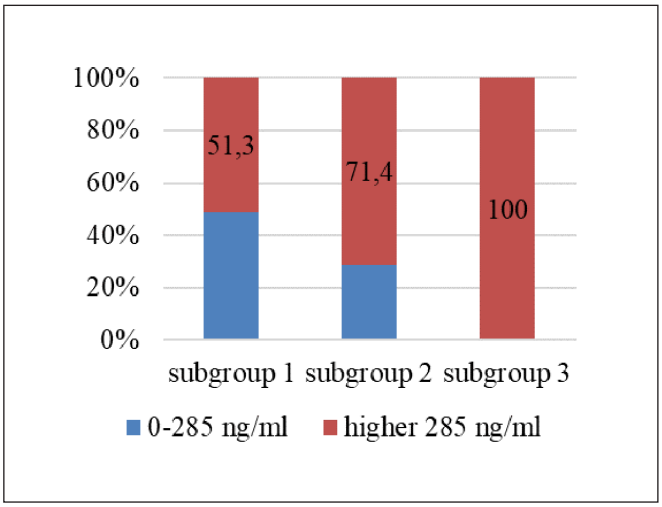

Fig. 1. Levels of D-dimer in main group on visit 1

All patients gave informed consent to conduct the necessary research methods.

The study was conducted according to Helsinki declaration with the permission of the Bioethical committee of the XXXX University (Approval No 2021/10-26).

RESULTS

Common clinical manifestations in mild patients included rhinorrhea, sore throat, anosmia, and fever. Patients with lung damage were more likely to have cough, shortness of breath and chest pain. Dyspeptic and cardiovascular manifestations occurred with approximately the same frequency in all subgroups, and the most common symptoms for all patients there were general inflammatory signs. The majority of patients with critical COVID-19 showed varying degrees of impaired consciousness and edema, which may be a manifestation of multiorgan changes in this category of patients. Vital signs in the main group on visit 1 are presented in Table I.

Regarding coagulation parameters, which are presented in Table II, an increase in PT was observed in only 41 (31,5%) patients (5 – with a critical course, 22 – with a severe course, 14 – with moderate course).

The level of fibrinogen was higher than the reference values in more than half of the examined patients (69 (53,1%)). The mean value differed significantly between subgroups, and its maximum values were observed in patients with a critical course (in three of them (50%) the level of the indicator exceeded the diagnostic maximum (7,5 g/l)).

The level of D-dimer was significantly elevated in the examined patients and correlated with the severity of the disease (Fig.1). However, in all patients of subgroup 3 it exceeded 2000 ng/ml, and in two people it was even higher than the diagnostic maximum (7500 ng/ml), due to which it was technically impossible to determine its true level.

A significant difference between the subgroups was observed in the number of points on the scales ISTH DIC and Padua. The maximum number of points was obtained by patients of subgroup 4 (Table II), which indicates the maximum risk of thrombotic complications, as well as DIC.

To determine the factors that could be recognized as risk markers for the progression of COVID-19-associated pneumonia, we conducted a ROC analysis. Clinical (levels of RR, SpO_2, BMI, body temperature), laboratory (D-dimer, fibrinogen) as well as integral scores (PPS, IDTH DIC) at admission were marked as variables and as the classification grouping was the fact of progression of pathology.

The analysis showed that among selected clinical indicators only the RR at admission was the sensitive and specific factor associated with the progression of the clinical course (Fig. 2), and the cut-off criterion is the RR more than 20 per 1 minute.

Among coagulation parameters the most sensitive and specific factors associated with the progression of the clinical course is the D-dimer (AUC = 0.837, p <0.001) at admission (Fig. 3), and the cut-off criterion is its level 259 ng/ml (Fig. 4), which is formally within the currently known reference values of this indicator.

Regarding other indicators, fibrinogen (AUC=0.589, p = 0,08) did not prove to be a predictor of the disease at a reliable level, while the scales DIC (AUC=0.846, p<0.001) and Padua (AUC=0.755, p<0.01) were highly specific and sensitive indicators that can predict the progression of COVID-19 (Fig.3). Moreover, the cut-off criteria more than 2 points on the DIC scale and 4 points on the Padua scale.

DISCUSSION

The are many authors who showed that COVID-19 related thrombosis can affect multiple organs of the body, presenting in the form of arterial or venous thrombosis such as ischemic stroke, myocardial infarction, mesenteric ischemia, limb ischemia, deep vein thrombosis (DVT), or a pulmonary embolism (PE). DVT and PE has an overall incidence of 6–26%, and severely ill COVID-19 patients have even higher incidence of thromboembolism. On the other hand, incidence of arterial thromboembolism is much lower with incidence of 0.7%–3.7%. D-dimer is found to be an independent risk factor, and IMPROVE score, Caprini score, and Padua score have all been used as predictors. [13, 14]

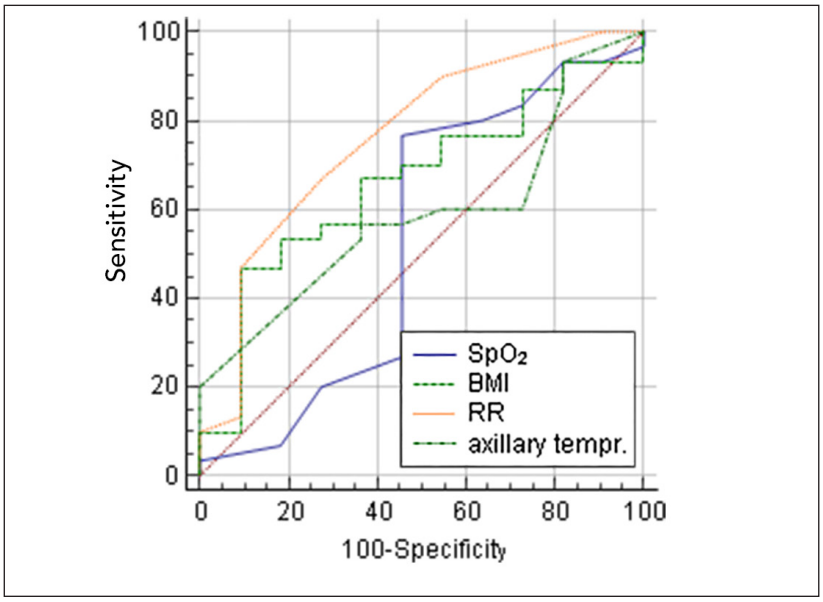

Fig. 2. ROC-analysis of clinical parameters like predictors of COVID-19 progression

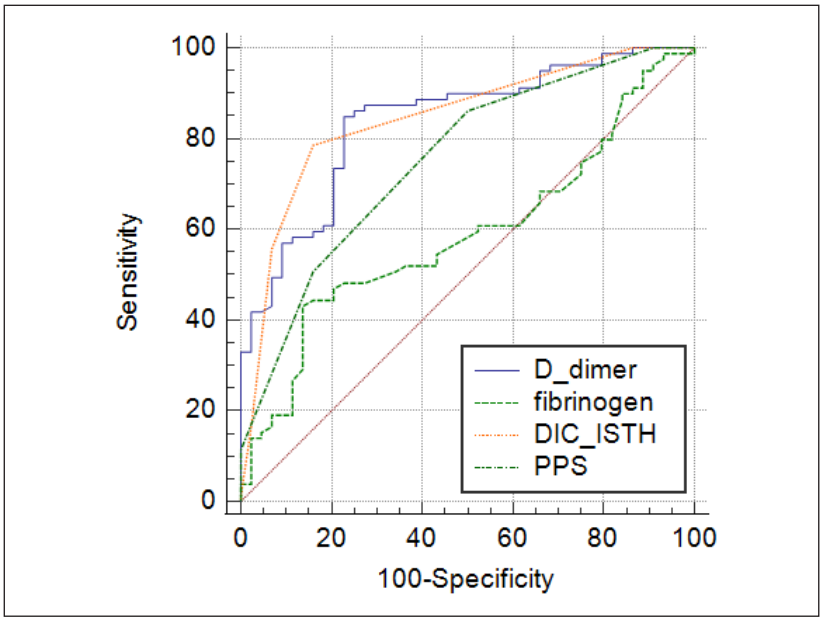

Fig. 3. ROC-analysis of coagulation parameters like predictors of COVID-19 progression

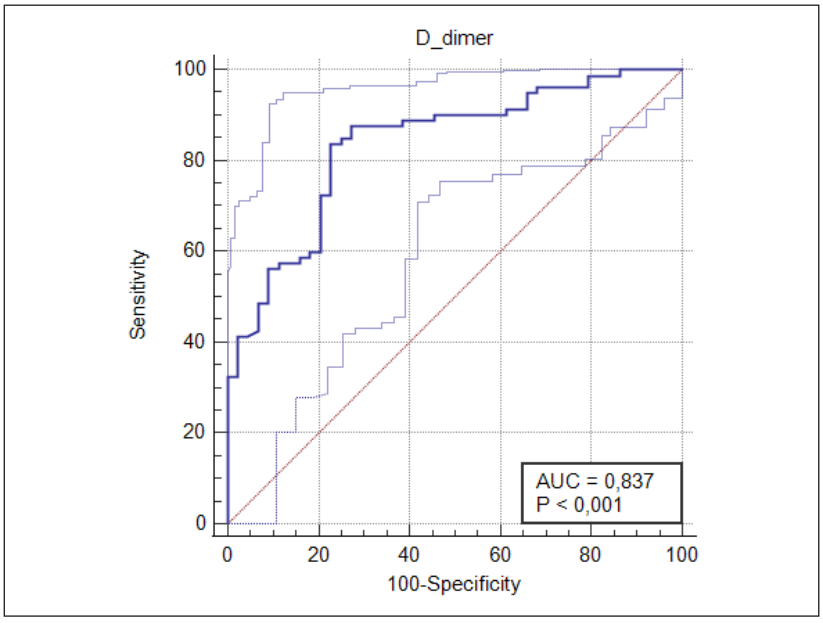

Fig. 4. ROC-analysis of D-dimer like predictor of COVID-19 progression

Clinical studies support the monitoring of D-dimers in COVID-19 patients and describe its correlation with the unfavorable evolution of the cases with increased D-dimers values. [14] In our research we get that the level of D-dimer above 260 ng/ml is associated with 3 times higher risk of COVID-19 progression (Odds ratio=3,66, p=0,043). It means that for COVID-19 our well-known references should be revised, because we are faced with vessels microthrombosis, but not always with massive pulmonary embolism.

A study of 2377 adult patients hospitalized with COVID-19 was performed on patients with D-dimers > 2000 ng/mL and observed a risk of critical illness of 66%, thrombotic event of 37.8% [15].

Another meta-analysis of 16 clinical trials showed higher levels of D-dimers in patients with severe disease and death from COVID-19 and noted the usefulness of anticoagulant therapy with lower mortality in patients treated with anticoagulants than those without anticoagulant therapy. [16]

DIC scale and the Padua scale demonstrated high specific and sensitive indicators that can predict the progression of COVID-19 during hospitalization. Wenyu Wang reported that D-dimer screening and Padua score assessment is helpful in the prevention of thrombosis. It may be more beneficial to perform early Doppler ultrasound of the lower limbs in every patient with critical COVID-19. [17]

Clinical warnings and limiting factors that could potentially limit our findings are uncertainty about whether antiplatelet and anticoagulant therapy was used in the outpatient phase prior to inclusion in the study; relatively small number of patients, one-centered study.

CONCLUSIONS

Thus, the determination of D-dimer, fibrinogen, as well as the calculation of the number of points on the DIC and Padua scales is a useful tool not only to estimate the severity of COVID-19, but also to predict the prognosis. Thus, the level of D-dimer above 260 ng/ml, as well as the presence of three or more points on the DIC scale and/or five or more points on the Padua scale in patients with COVID-19-associated pneumonia during hospitalization significantly increases the risk of progression clinical process.

MAIN POINTS

1. D-dimer, DIC and Padua scales are useful tools not only to estimate the severity of COVID-19, but also to predict the prognosis.
2. D-dimer at admission higher 260 ng/ml (which is formally a norm) increases the risk of COVID-19 progression in 3 times.
3. For COVID-19 the well-known D-dimer references should be revised, because we are faced with vessels microthrombosis, but not massive pulmonary embolism.
4. More than 2 points by the DIC scale and 4 points by the Padua scale are highly specific and sensitive predictors of the COVID-19 progression.

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ORIGINAL ARTICLE

TREATMENT OPTIMIZATION IN MANAGEMENT OF COMBINED PATHOLOGY – ARTERIAL HYPERTENSION AND POST-COVID SYNDROME IN ELDERLY PATIENTS

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ABSTRACT

The aim: Purpose of the study. Our research is aimed at the increase in the treatment effectiveness for combined pathology, namely, arterial hypertension (AH) and post-COVID syndrome in elderly patients at the stage of providing medical care by family medicine general practitioners with the use of statins, antiplatelet agents, as well as endothelial-protective drug – L-arginine and anxiolytic effect – mebicar against the background of basic antihypertensive therapy.

Materials and methods: The study included treatment and observation of 50 elderly patients with hypertension and post-COVID syndrome. The average age was 68.7±1.89 years.

Results: The use of mebicar with moderate tranquilizing (anxiolytic) effect and endothelium-protector – L-arginine in the comprehensive treatment of elderly patients with combined pathology – AH and post-COVID syndrome contributed to the elimination of the main clinical symptoms (headache, poor sleep) in a shorter time; provided significant decrease in the level of systolic blood pressure, reactive anxiety, the decrease in total blood cholesterol, and improvement in blood rheology.

Conclusions: Treatment optimization for combined pathology – arterial hypertension and post-COVID syndrome in elderly patients with the use of L-arginine and mebicar in comprehensive treatment, improves the quality of patients' life, reduces the treatment duration.

KEY WORDS: arterial hypertension, L-arginine, post-COVID syndrome, mebicar

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INTRODUCTION

Despite the significant progress in understanding epidemiology, pathophysiology and risks associated with the increased blood pressure, arterial hypertension (AH) remains a serious global problem [1]. The prevalence of hypertension in people of retirement age exceeds the average by 1.8 times [2,3]. According to the World Health Organization, the number of elderly and senile people will increase to almost 40% over the next decades [4,5]. At the same time, coronavirus disease (COVID-19) is the extremely serious problem in connection with pandemic in the world. The causative agent of coronavirus infection (COVID-19), as a rule, severely affects elderly patients over 65 years of age [6]. Moreover, patients after the acute phase of COVID-19 present with the signs of chronic disease exacerbations, the increase in the severity of functional and morphological disorders experienced before the onset of coronavirus infection [7]. The relevance of the problem consists in unclear understanding the ways and approaches in diagnosis and management of post-COVID syndrome [7]. Post-COVID syndrome is characterized by complaints of constant fatigue, anxiety and weakness. More often post-COVID syndrome manifests in the form of lung damage, mental disorders and asthenia [8-10]. Currently, the treatment of post-COVID syndrome in patients with cardiovascular pathology becomes the urgent issue, especially in the family medicine practice. The main pathomorphological process and the trigger mechanism of post-COVID syndrome is endotheliitis, resulting from direct infection of endothelial cells by SARS-CoV-2 virus (or indirect – by cytokines and free radicals through immune responses) which causes the development of generalized endothelial dysfunction, that in turn disrupts microcirculation, vasoconstriction and leads to the further development of organ ischemia, inflammation and tissue edema, transition of the smoldering systemic inflammation to post-COVID syndrome [8,10]. AH destroys the architectonics of endothelial cells followed by the increased production of vasoconstrictor endothelin-1 and vascular remodeling

with the decrease in vascular elasticity [11]. It should be noted that L-arginine (Tivortin aspartate) not only improves the condition of the endothelium, but also reduces the manifestations of systemic inflammation and oxidative stress, the formation of post-COVID syndrome. [8]. L-arginine is a conditionally essential amino acid, which is an active and multifaceted cellular regulator of many essential functions in the organism, plays antihypoxic, membrane stabilizing, antioxidant and detoxification actions [12]. Currently, it has also been proven that morbidity and mortality from cardiovascular diseases are largely associated with psychological factors, in particular, a significant prevalence of depression and anxiety symptoms has been detected among patients with AH [13]. The data of recent studies suggest that long-term psychological stress caused by activation of the hypothalamic-pituitary-adrenal axis, low-intensity inflammation, leads to endothelial dysfunction and steady increase in the blood pressure (BP) [13]. According to research data, in patients with arterial hypertension, the frequency of anxiety disorders was 1.5 times higher than in patients with normal blood pressure [1]. Therefore, the comprehensive treatment of such patients should include drugs with tranquilizing effect which reduce the anxiety. In clinical practice, they use mebicar (adaptol) for astheno-vegetative syndrome elimination. It has moderate tranquilizing (anxiolytic) effect, relieves the feelings of anxiety, fear, internal emotional stress and irritation [14].

THE AIM

Purpose of the study. Optimization of treatment and improvement of the quality of life of elderly patients with combined pathology – arterial hypertension (AH) and post-covid syndrome by general practitioners of family medicine with the use of additional drugs – with the endothelium-protective effect of L- arginine and mebicar with a sedative effect.

MATERIALS AND METHODS

The research presented includes observation and treatment of 50 elderly patients for hypertension with post-COVID syndrome. The average age was 68.7±1.89 years. The diagnosis was verified on the basis of complaints, anamnesis data (coronavirus disease (COVID-19) experienced over the past 3-6 months), physical examination, general clinical and laboratory-instrumental methods (complete blood count, ECG, echocardiography CS, CRP, coagulograms, lipidograms, urea, residual nitrogen). The study included elderly patients experiencing hypertension with post-COVID syndrome with preserved ejection fraction (EF) of the left ventricle (40%). All patients underwent a preliminary screening test for SARS-CoV-2 antigen using rapid tests for SARS-CoV-2 antigen, a negative result was obtained, which ruled out recurrence of COVID-19. The psychological state of elderly patients with comorbid pathology – hypertension and COPD – was assessed with Ch. D. Spielberger – Yu. L. Khanin questionnaire [14,15]. When interpreting the test results, they use the following scores: less than 30 points – low anxiety; 31-45 points – moderate anxiety; 46 or more points – high anxiety.

The life quality assessment at the beginning and during the treatment was conducted applying SF-36 questionnaire which is considered the "gold standard" [3]. The SF-36 questionnaire has 3 levels: 1) questions; 2) 8 scales; 3) 2 total measurements combining the scales. There are 36 questions in the questionnaire, 35 of which are used to process scores according to 8 scales, which are grouped into two general indicators: "Physical component of health", including scales: General health (GH); Physical functioning (PF); Role Physical Functioning (RP); Pain intensity (Bodily pain – BP); and "Psychological component of health", including scales: Mental Health (MH); Vitality (VT); Role functioning caused by emotional state (Role-Emotional – RE); Social Functioning (SF) [16]. Each scale has a different number of questions. The indicators of each scale fluctuate between 0 and 100 points (relative units), where 100 represents full health. One question of the questionnaire (number 2), concerning comparison of the health state at a given time with the previous year, does not belong to any scale and is assessed separately. The SF-36 questionnaire makes it possible to quantify QoL according to the indicated scales [3].

The patients were distributed into two groups: the first – control group (n=25) – was prescribed comprehensive basic antihypertensive therapy, statins, antiplatelet agents (telmisartan/amlodipine, rosuvastatin, cardiomagnyl). The second group – the main group (n=25) was prescribed the basic therapy and, additionally, solution of L-arginine 4.2%, 10 ml 2 times a day as well as mebicar 500 mg, 1 tablet 2 times a day. The groups were age and gender comparable. The observation period lasted 1 month.

The survey data were processed considering the special algorithm developed for assessing QoL according to SF-36. These data are presented in the middle and standard pardon of the middle. In order to directly establish the nature of the interrelationship, we performed a correlation analysis between groups of inde-

Table I. Clinical indicators in patients of the main and control groups

Indicators	Control group		Main group	
BP systolic mm.Hg	179.8±1.52*	134.1±1.23	177.9±1.02*	128.6±1.28**
BP Diastolic mm.Hg	95,13±3.22*	76.0±3.7	95,27±3.24*	69.6±4.33
RA points	48.4±1.24*	42.93±1.13	48.13±1.36*	39.22±1.41**
APTT sec.	19.87±1.01	22.96±2.01	19.64±0.86*	25.49±1.81
Total cholesterol mmol/l	4.83±0.4	3.97±0.22	4.75±0.30*	3.81±0.17

* p<0.05 – differences are significant between groups of patients before and after treatment;
** p<0.05 – differences are significant between the patients of the main and control groups after treatment.

Table II. Indicators of the quality of life of elderly patients with AH and post-COVID syndrome (main and control groups)

Questionnaire scales SF 36	Control group		Main group	
	Before treatment	After treatment	Before treatment	After treatment
1. General health – GH	24,07±2,52*	36,17±3,17	25,53±2,38*	39,6±4,01
2. Role-Physical Functioning – RP	24,73±2,12*	37,63±2,68	24,84±2,53*	40,13±3,17
3. Physical Functioning – PF	22,27±4,55*	35,6±3,64	26,07±4,02*	38,79±3,49
4. Bodily pain – BP	24,73±3,195*	35,33±1,98	26,73±2,98*	42,73±2,31**
5. Vitality – VT	24,53±3,48*	36,93±2,34	25,67±2,58*	43,22±2,17
6. Role-Emotional – RE	25,47±2,97 *	37,53±2,85	26,3±1,97*	41,82±2,12
7. Mental Health – MH	27,4±3,01*	35,13±2,13	26,93±2,98*	44,6±3,04**
8. Social Functioning – SF	28,27±4,16	37,07±1,71	26,47±2,72*	42,51±1,95**

*p<0.05 – differences are significant between groups of patients before and after treatment;
** p<0.05 – differences are significant between the patients of the main and control groups after treatment.

pendent samples with a match coefficient Spearman correlations. statistical significance intergroup viability was assessed using the Mann-Whitney method. Statistical analysis were carried out with the help of the program «Statistica 6.0» (StatSoft Inc., USA, serial No. RGXR412D674002FWC7). For all types of analysis, the statistically significant values were taken into account with equal significance less than 0.05.

RESULTS

The comparative analysis of the main clinical and laboratory parameters determined that patients of the main group who received L-arginine and mebicar against the background of basic therapy had significant differences in the timing of headache subsiding, sleep improving, decrease in BP as well as the level of reactive anxiety (RA), decrease in the level of total cholesterol in the blood (p<0.05) compared with the control group of patients. Thus, the average time for headache subsiding in patients of the main group was 6.4±0.79 days versus 8.83±0.71 days in patients of the control group (p<0.05); sleep normalization – 7.0±0.73 days versus 9.3±0.77 days in patients of the control group (p<0.05). The data obtained convincingly indicate that the comprehensive antihypertensive therapy which includes the use of L-arginine and mebicar contributed to a more rapid elimination of clinical manifestations when managing the combined pathology – hypertension and post-COVID syndrome in elderly patients.

Patients of the main group had significant decrease in systolic blood pressure (SBP) compared with patients in the control group (p<0.05). Thus, in patients taking L-arginine and mebicar in addition to treatment, SBP decreased by 32.4% (from 177.9±3.98 to 120.3±1.79 (p<0.05), while in patients of the control group – by 29.9% (from 179.8±5.9 to 126.1±2.29 mm Hg (p<0.05). Diastolic blood pressure (DBP) decreased by 27% in the main group patients (from 95.27 ± 3.24 to 69.6±4.33 (p<0.05), while in patients of the control group – by 20.11 % (from 95.13±3.22 to76.0±3.7) (p<0.05). The research data indicate that the comprehensive therapy which includes L-arginine contributed to the increase in endothelium-dependent vasodilation, which coincides with the authors' opinion [13,16].

The examination data determined that the elderly patients with hypertension and post-COVID syndrome presented with the high level of reactive anxiety (RA), which changed during treatment. The main group patients

developed more significant decrease in RA ($p<0.05$) during the treatment compared with the control group patients. Thus, in the main group patients, the level of RA decreased by 18.6% (from 48.13±1.36 to 39.22±1.41) ($p<0.05$) ($p<0.05$), while in the control group patients – by 11.7% (from 48.4±1.24 to 42.73±1.48) ($p<0.05$). The data obtained make it possible to state that reactive anxiety is a very mobile trait and is characterized by reversibility upon normalization of the somatic state of a person [17].

The main group patients developed significant increase in APTT level after therapy, namely, by 29,7% (from 19.64±0.86 before treatment; 25.49±1.81 – after therapy) ($p<0.05$), which indicates the improvement in blood rheology and a positive effect on the anticoagulant system parameters. While in the control group patients, APTT increased insignificantly by 11.5% (from 19.87±1.01 to 22.96±2.01) ($p>0.05$).

After treatment, the main group patients presented with significant decrease in the level of total cholesterol by 19,8% (from 4.75±0,30 mmol/l to 3.81±0,17 mmol/l) ($p<0.05$), while the cholesterol level did not decrease significantly in the control group patients, namely, by17.81% (from 4.83±0.4 to 3.97±0.22) ($p>0.05$). The decrease in cholesterol level ($p<0.05$) indicates the positive effect of L-arginine on lipid metabolism, which coincides with the scientists' view [18]. The data of research are presented in the Table I.

Considering that the ultimate goal of any therapy is to increase the life expectancy of patients and improve its quality, we studied QoL of patients before and after the course of therapy. The data obtained are presented in Table II.

DISCUSSION

After comprehensive treatment, compared to the control group, patients in the main group showed a significant increase in indicators, according to the following scales: Pain intensity (Bodily pain – BP), Mental Health (MH), Social Functioning (SF). ($p<0.05$), which indicates a significant improvement in the quality of life in this category of patients.

There were correlative dependences between the levels of systolic BP and RA in the main group patients ($r=0.392$; pbefore treatment, which proves the expediency of applying the drug with a moderate tranquilizing (anxiolytic) effect – mebicar and endothelial protector – L-arginine in the comprehensive treatment of elderly patients with combined pathology – hypertension and post-COVID syndrome.

Thus, comprehensive examination of elderly patients with combined pathology – hypertension and post-COVID syndrome using questionnaires – Ch.D. Spielberger – Y.L. Khanin, and the SF-36 questionnaire, allows to assess the level of reactive anxiety, the quality of life in this category of patients, with the aim of developing a rational complex therapy.

Given the important role of endothelial nitric oxide production disorders in the pathogenesis of hypertension, the feasibility of using drugs that are its donors is justified. Such drugs include L-arginine [19,20]. L-arginine can be recommended for the purpose of: normalizing high blood pressure; prevention of the formation and development of atherosclerotic plaques and normalization of elevated cholesterol levels; protection of the heart and blood vessels in conditions of oxidative stress; reducing the risk of thrombosis; maintenance of normal blood circulation and oxygen supply to various organs and tissues; general strengthening of the body and improvement of immunity; improvement of microcirculation in the tissues of the central nervous system, which enhances the metabolism in neurons, contributes to the improvement of cognitive functions – memory, attention, mental activity [19] .

The results of the conducted research allow us to recommend L-arginine in the complex treatment of elderly patients with combined pathology – hypertension and post-covid syndrome.

At the same time, in the treatment of psychosomatic disorders and the normalization of the body's adaptive capabilities in a wide range of pathological conditions, according to the data of scientists, the use of the drug mebikar is justified. According to the results of the authors' research [14, 21] mebikar helps to reduce anxiety symptoms, it also has an antihypertensive effect, which makes it possible to recommend mebikar in the complex treatment of elderly patients with combined pathology – hypertension and post-covid syndrome.

The results of the conducted studies show that the optimization of the treatment of elderly patients with combined pathology – arterial hypertension and post-covid syndrome with additional use of drugs – L-arginine and mebikar use on the background of basic therapy contributed to the improvement of the quality of life of patients and due to:

- elimination of the main clinical manifestations in a shorter time: (the average duration of headache cessation in patients of the main group was 6.4±0.79 days, against 8.83±0.71 days in patients of the control group ($p<0.05$) average terms of normalization of sleep – 7.0±0.73 days, against 9.3±0.77 days in patients of the control group ($p<0.05$);
- more significant decrease in the level of systolic blood pressure by 32.4%, (while in patients of the control group – by 29.9%) ($p<0.05$).

– more significant reduction in the level of reactive anxiety by 18.6%, (while in patients of the control group – by 11.7%) ($p<0.05$),
– a significant increase in the level of PT – by 29.7% ($p<0.05$), a significant decrease in the level of total cholesterol by 19.8% ($p<0.05$), while in patients of the control group these indicators improved without statistical significance.

Positive clinical dynamics in patients who received complex therapy with the use of L-arginine and mebicar contributed to shortening the duration of treatment by 1.8 days.

CONCLUSIONS

Optimization of treatment of combined pathology – arterial hypertension and post-covid syndrome in elderly patients with additional use of L-arginine and mebicar drugs to the basic therapy contributes to the improvement of the quality of patients life, due to the regression of the main clinical manifestations of the disease in a shorter time, a significant decrease in the level of blood pressure and reactive anxiety, shortening the period of treatment of patients.

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ORIGINAL ARTICLE

CLINICAL FEATURES OF THE CARIES COURSE OF TEMPORARY TEETH IN CHILDREN

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ABSTRACT

The aim: Assess the incidence of caries of temporary teeth in children using the indices drxfm, SIC, ICDAS.

Materials and methods: A survey of 255 children 3 and 6 years old was conducted and 2 research groups were formed. We determined the prevalence, intensity of caries by the expanded formula drxfm, SIC-index and ICDAS II 1-6, as well as determined the Green-Vermilion hygienic index.

Results: The high prevalence of caries of permanent teeth according to WHO criteria was revealed. The average value of ICDAS II 1-6 at the age of 3 years is 1.91 ± 0.24 teeth, $p < 0.05$. In 6-year-olds, this index was 3.78 ± 0.32 teeth, $p < 0.05$. In 3-year-old children, the average value of SIC was 2.64 ± 0.09 teeth, which probably differed from that of 6-year-old children – 6.82 ± 0.12 teeth. Regarding the Green-Vermilion index, unsatisfactory oral hygiene was found in children aged 3 and 6 years.

Conclusions: Thus, the obtained high rates of prevalence and intensity of caries determine the special importance of caries prevention measures and indicate the need to find new approaches in the fight for dental health of children.

KEY WORDS: dental health, caries, ICDAS, drxfm

Wiad Lek. 2023;76(7):1549-1553

INTRODUCTION

Undoubtedly, the most common disease of the oral cavity in childhood is caries. This dental nosology is one of the oldest and most common in the world [1, 2].

The results of epidemiological studies show that a high prevalence of caries of temporary teeth is observed in children under two years – 62% of respondents, at three years – 70.3% [3, 4]. In different regions of Ukraine this indicator remains consistently high, namely: in children of Prykarpattia – 90.95 ± 2.66%, Zakarpattia – 98%, Lviv region – from 78 to 93%, Poltava region – 50 + 10%, Vinnytsia region – 85, 97 ± 1.90%, Odessa region – 63.8% [2-6]. This indicates the need to start preventive measures from an early age of the child, taking into account the regional features of the formation of dental pathology [3, 7].

Factors in the development of dental caries are individual. There are more than 100 causes of the risk of dental caries in childhood. They can be of different intensity and nature, there are different options for their interaction [8, 9]. The prevalence of caries of temporary teeth depends on the socio-economic status of the family, environmental factors, fluoride exposure, dental prophylaxis, and is determined by the composition and structure of enamel and other tooth tissues, specific and nonspecific oral protection factors, quantitative and qualitative indicators of oral fluid, the presence of bad habits, the properties of plaque, and all this depends on the general condition of the body [5, 10].

THE AIM

Assess the incidence of caries of temporary teeth in children using the indices drxfm, SIC, ICDAS.

MATERIALS AND METHODS

For this purpose, clinical examinations of 255 children aged 3 and 6 (121 boys and 134 girls) living in Bukovina were conducted. Dental examination of children was carried out on the clinical basis of the Department of Pediatric Dentistry at the Municipal City Institution «Regional Children's Clinical Hospital» (Chernivtsi, Ukraine). These children were divided into 2 age groups: I – 3 years; II – 6 years. Evaluation of dental hard tissues was performed by determining the prevalence and intensity of carious lesions of the teeth according to the dmf index and its modification (Alimsky et al, 2007). Of particular importance is the detailed characteristics of the element «d», which, in turn, is divided into: super-

Table I. Intensity of caries of temporary teeth in children according to the ICDAS II index, points

ICDAS II	I group	II group
ICDAS II $_{1-6}$	1,91±0,24	3,78±0,32*
ICDAS II $_{4-6}$	0,63±0,19	1,63±0,22*
1	0,39±0,08	1,07±0,18*
2	0,33±0,13	1,03±0,23*
3-4	0,82±0,09	1,19±0,18*
5	0,23±0,03	0,20±0,02*
6	0,14±0,04	0,29±0,12
R	-	0,12±0,03
X	-	0,02±0,01
F	0,43±0,17	1,39±0,37*
M	-	0,06±0,01

Notes. *: p – the difference between the indicators of children 3 and 6 years is probable (p <0,05).

ficial and secondary caries (actually the element «d»), complications of caries to be treated (element «r») and complications of caries subject to removal (element «x»). Element «f» – filled teeth, «m» – removed.

At any intensity of dental caries, as assessed by the «dmf» index, an additional determination of SiC-index was performed in 1/3 of the examined group of children with the highest individual indicators of the index.

In addition to the «dmf» index, we assessed caries of permanent teeth according to the International Caries Determination and Assessment System – ICDAS. ICDAS II criteria (1-6) were used to compare the incidence of caries in permanent teeth.

In order to objectify the state of oral hygiene, all patients underwent determination of the Green Vermilion oral hygiene index.

The degree of probability of the obtained results was statistically assessed in the case of normality of the distribution of both samples according to the Student-Fisher test, in other cases – U-Wilkokson for independent samples and T-Wilkokson criterion for dependent samples.

RESULTS

According to the results of the study of the state of hard tissues of temporary teeth in children of both groups revealed a high prevalence of caries according to WHO criteria (Fig. 1), while the number of healthy teeth (without caries) ranged from 35.72 to 52.94%.

Analysis of the intensity of carious lesions of temporary teeth by depth (according to the ICDAS system) found that its average value at the aged 3 is 1.91 ± 0.24, while the index ICDAS II 4-6 – 2.05 times less – 0, 63 ± 0.19 teeth, p <0.05 (Table I). The values of both indices increase with age. Thus, the average value of ICDAS II 4-6 increases up to 6 years by 54.18% and is 2.03 ± 0.22 teeth, p <0.05. A probable increase to 4.94 ± 0.32 teeth is observed relative to the ICDAS II index 1-6. As for the structure of carious lesions of temporary teeth, in 35.19 ± 1.92% of cases the middle layers of dentin are affected (code 3-4), with the same frequency at the age of 3 years and 6 years. Caries of deep dentin layers (code 5-6) was found in 33.18 ± 2.27% of teeth, mostly at the age of 6 years, which indicates the lack of preventive measures and untimely treatment of caries of temporary teeth in children. Carious lesions of enamel (code 1-2) were observed in smaller quantities – in 31.69 ± 2.21% of teeth.

The initial stage of dental caries is described by 2 codes of the ICDAS II index, namely: code 1 is characterized by visible changes on the enamel surface only after prolonged drying, and code 2 – obvious visual changes of enamel. We found a probable increase in the number of affected teeth with codes 1 and 2 with age. Thus, the number of carious lesions of temporary teeth under code 1 from 3 to 6 years increases from 0.39 ± 0.08 teeth to 1.22 ± 0.18 teeth, p <0.05.

The number of carious cavities with visible localized destruction of the enamel without signs of dentin damage (ICDAS code 3) is likely to increase per one child with age, but their number with code 4 (ICDAS II code) increases slightly. The same applies to deep carious lesions (ICDAS II code 5 and code 6).

Component «r», ie complications of caries that require treatment, and the structure of «x», the presence of carious teeth to be removed in children aged 3 years are absent. For 6-year-old children, the availability of such data indicates obvious problems not only in the primary prevention of caries, but also in timely quality treatment.

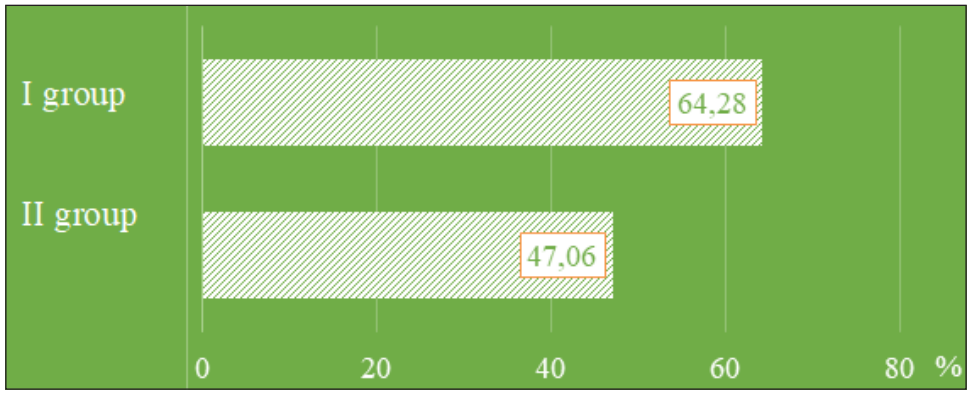

Fig. 1. Prevalence of caries of temporary teeth in children.

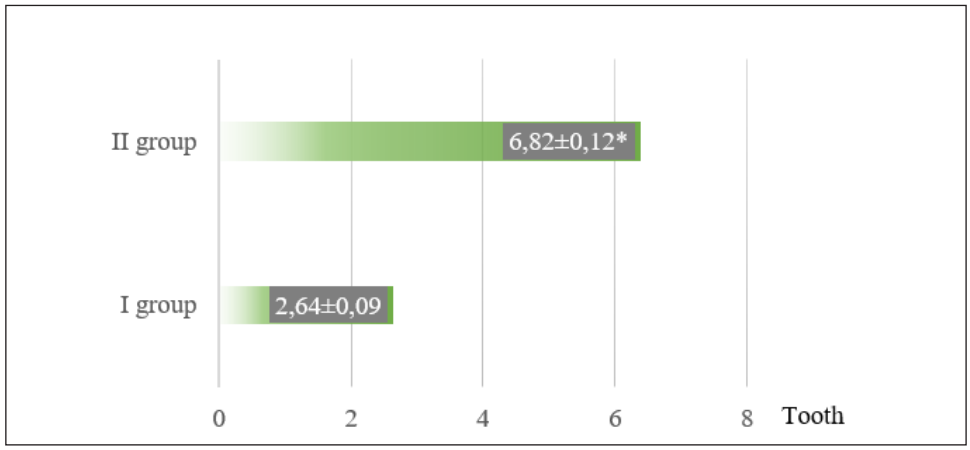

Fig. 2. SiC-index in children.
Notes. p – the difference between the indicators of children 3 and 6 years is probable (p <0,05).

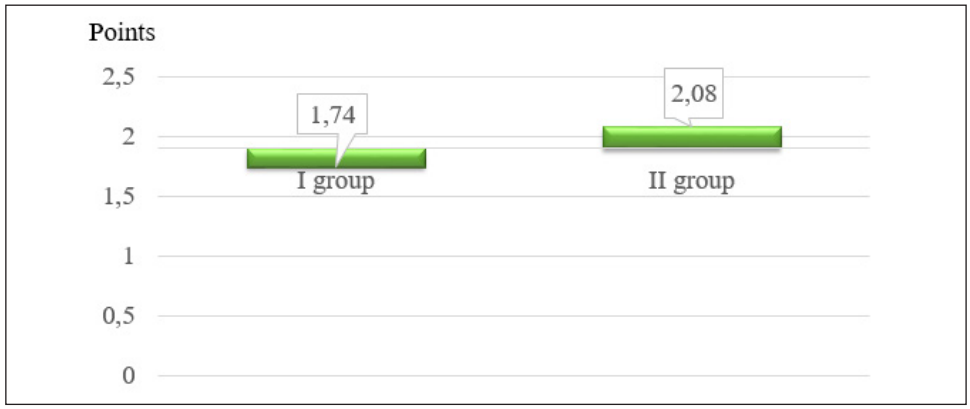

Fig. 3. Green Vermilion index in children.
Notes. p – the difference between the indicators of children 12 and 15 years is probable (p <0,05).

We note a probable increase in the component «f» in children with age, namely from 0.43 ± 0.39 in 3-year-olds to 1.89 ± 0.37 in 6-year-olds. The predominance of the component «d» over «f» indicates the lack of treatment and prevention measures in children of these age groups.

It is believed that the indicator of the quality of dental care for children is the component «m» in the formula dmf. As it is known, according to WHO recommendations, children and adolescents under 18 should not have permanent teeth removed. In our study, the average number of removed teeth in the examined 6-year-old children was 0.02 ± 0.01. The highest intensity of dental caries in children according to the SiC-index in young children was 2.64 ± 0.09 teeth, which probably differed from that of 6-year-old children – 6.82 ± 0.12 teeth (Fig. 2).

The values of the Green Vermilion index reflect in children aged 3 and 6 years of unsatisfactory oral hygiene, but the indicators in both groups were significantly different (p<0.05) (Fig. 3).

DISCUSSION

In bibliosemantic research it found a high prevalence of caries of temporary teeth in children 2, 3, 4, 5 years in different regions of Ukraine, which, in particular, coincides with our indicators [5].

In another research we found that in children aged 3 32.42% of all teeth that present in the oral cavity are

carious, which is probably different from our findings. We associate this difference with the living conditions of children in Bukovina in the iodine deficiency zone [3].

The highest prevalence of caries of temporary teeth was found in children 3-4 years compared to children 4-5 or 5-6 years, which differs from our indicators. Regarding the depth of the lesion, we have the same tendency as author [11].

Prevalence of caries of temporary teeth in 6-7 year old children is 87.3% and a caries intensity is 2.75 [12]. Studies of the prevalence and intensity of caries in different regions of Ukraine, including our results, indicate a high risk of caries with «mass» prevalence and «high» intensity of caries of both temporary and permanent teeth [5, 9, 12].

The researchers found high prevalence and intensity of dental caries in children living under conditions of biogeochemical deficient of trace elements [6]. Given that Bukovina is an iodine deficiency zone, it is interesting to compare indicators. In 6-year-old children living in Zakarpathia region, the prevalence of caries ranged from 88.6 to 96.4%, which is probably higher than ours. The authors also noted a significant difference in the performance of children in the presence of somatic pathologies and stress [6].

CONCLUSIONS

Thus, we found high rates of caries of temporary teeth in children aged 3 and 6. Features of clinical manifestations of caries in children aged 6 are compared with 3 years old: high prevalence and intensity of caries by WHO criteria (ICDAS II index 1-6 is 1.97 times higher, and SIC-index – 2.58 times), the presence components «m», «r», «x» in the structure of drxfm and unsatisfactory level of oral hygiene (Green Vermilion index is in 16.34% higher).

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

STUDY OF IMMUNE-INFLAMMATORY RESPONSE CHANGES IN ORAL FLUID IN PATIENTS WITH DISEASES OF PERIODONTAL TISSUES IN COMBINATION WITH GENERAL SOMATIC PATHOLOGY

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ABSTRACT

The aim: To determine the concentration of markers of the immune-inflammatory response (IL-1β, IL-10, IL-1β / IL-10, hsCRP) in oral fluid in patients with diseases of periodontal tissues in combination with general somatic pathology.

Materials and methods: The study was conducted at Danylo Halytsky Lviv National Medical University, Department of therapeutic dentistry FPGE, Lviv, Ukraine. The patients were divided into two groups: the main group – 144 patients (with periodontal tissue diseases on the background of general somatic pathology) and the control group – 30 somatically and dentally healthy persons, in whose oral fluid was determined the concentration of IL-1β, IL-10, hsCRP by the enzyme immunoassay method.

Results: As a result of our research, it was found that in people with periodontal tissue diseases, against the background of general somatic pathology, there is an activation of the immune-inflammatory response, which aggravates the course of general somatic and dental diseases in this contingent of patients.

Conclusions: Therefore, in patients with periodontal tissue diseases on the background of somatic diseases, a significant increase in the level of the pro-inflammatory cytokine IL-1β and hsCRP was determined against the background of a decrease in the anti-inflammatory cytokine IL-10 in the oral fluid compared to the values in the control group.

KEY WORDS: hsCRP, IL-10, IL-1β, oral fluid, periodontal tissue diseases

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INTRODUCTION

Pathology of periodontal tissues is a common phenomenon among the population of the entire globe, which represents a serious medical and social problem [1]. Ukraine is no exception: the prevalence of periodontal disease at the age of 45-50 reaches almost 90%, as evidenced by the results of research by domestic scientists, while many researchers note the high prevalence of periodontal tissue diseases in young people [2].

To date, it has been clearly established that inflammatory and dystrophic-inflammatory changes in periodontal tissues deepen when the body's resistance is reduced, caused by various factors: chronic debilitating diseases, stress and mental injuries, hormonal disorders, adverse environmental factors [3, 4].

Data from the literature show [5-7] that there are a number of diseases that are combined with an absolute regularity with periodontal disease: diabetes, hypertension, coronary heart disease, chronic diseases of the gastrointestinal tract, chronic obstructive pulmonary disease, rheumatoid arthritis, stressful situations.

According to the prevailing opinion of clinicians, the results of treatment of patients with periodontal tissue damage remain at an unsatisfactory level [8, 9]. Insufficient consideration of the impact of systemic diseases on the course of periodontal tissue diseases is one of the most likely reasons for its treatment [10]. All of the above determined the relevance of the topic and served as a basis for conducting research in this area with the aim of improving the diagnosis and treatment of periodontal tissue diseases against the background of general somatic pathology based on the obtained data.

THE AIM

To determine the concentration of markers of the immune-inflammatory response (IL-1β, IL-10, IL-1β / IL-10, hsCRP) in oral fluid in patients with diseases of periodontal tissues in combination with general somatic pathology.

MATERIALS AND METHODS

The study was conducted at Danylo Halytsky Lviv National Medical University, Department of therapeutic dentistry FPGE, Lviv, Ukraine. The object of the study was inflammatory, dystrophic-inflammatory and dystrophic processes in periodontal tissues at the patients with cardiovascular`s, neurological`s, gastroenterological`s, rheumatological`s diseases, who were receiving inpatient treatment at the Lviv Regional Clinical Hospital. Observation groups were formed depending on the nosological units of the disease, which were the most common in the department. So, in the department of cardiovascular surgery, the largest group was represented by patients with obliterating atherosclerosis – 36 patients; in the neurological department – 35 patients with vascular lesions of the central nervous system; in the gastroenterology department – 36 subjects with peptic ulcer disease and in the rheumatology department – 37 people with rheumatoid arthritis (main group). The obtained data were compared with the values 30 somatically and dentally healthy individuals (control group).

The classification of M. F. Danylevsky (1994) was used to assess the condition of periodontal tissues. All research results were registered in the medical record of the dental patient. The concentration of IL-1β, IL-10 and highly sensitive C-reactive protein (hsCRP) was determined in the patients' oral fluid. Oral fluid for laboratory tests was collected in the morning on an empty stomach, by spitting into measuring tubes, without stimulation [11]. Oral fluid samples were centrifuged at 3000 rpm for 15 minutes. The supernatant was examined. Determination of the levels of interleukin-1β, interleukin-10, hsCRP in oral fluid was carried out by the immunoenzymatic method [12, 13], with the help of original sets of reagents from the company "Vector Best" (Ukraine): "Interleukin-1-beta-UFA-Best", "Interleukin-10-UFA-Best", "CRB-UFA-Best (highly sensitive)", according to the instructions included with the set. The research was carried out on a tablet semi-automatic immunoenzymatic analyzer "SunriseTS", Austria, by the immunological "Sandwich" – reaction method [14].

The obtained results were statistically processed using the standard software package of «STATISTICA 6.0». The validity of the test data was determined using Student›s t-test [15].

RESULTS

As a result of the conducted studies, it was established that with inflammatory diseases of the periodontal tissues, the concentration of IL-1β in the oral fluid increased by 1.9 times in patients with rheumatological diseases, by 1.8 times in people with cardiovascular diseases, $p<0.05$, by 1.5 times in subjects with gastroenterological and by 1.3 times – with neurological diseases, $p>0.05$, in relation to the data in the control (Fig 1). At the same time, in patients with rheumatological diseases, the concentration of IL-1β in the oral fluid was probably higher than in people with neurological and gastroenterological pathologies, p_2, $p_3<0.01$.

At the same time, an increase in the concentration of hsCRP was noted in the oral fluid of the subjects: by 1.4 times – in persons with diseases of the cardiovascular system and with rheumatological lesions, $p<0.05$, and by 1.1 and 1.2 times – in patients with neurological and gastroenterological lesions, $p>0.05$, compared to the data in the control group. It should be noted that in patients with rheumatological diseases, the content of hsCRP in the oral fluid was significantly higher than in people with neurological and gastroenterological diseases, p_2, $p_3<0.01$.

The concentration of anti-inflammatory IL-10 in oral fluid decreased in inflammatory diseases of periodontal tissues: 1.6 times in patients with rheumatological disease, 1.5 times in patients with cardiovascular disease, $p<0.05$ and 1.2 times – in people with neurological and gastroenterological pathology, $p>0.05$, $p_1<0.05$, p_2, $p_3<0.01$.

We noted an increase in the ratio of concentrations of IL-1β / IL-10 in the oral fluid of the subjects: by 2.6 times in people with rheumatological diseases, by 2.2 times in patients with cardiovascular diseases, $p<0.05$, in 1.4 times in gastroenterological pathology and 1.2 times in people with neurological diseases. At the same time, the ratio of cytokines in the oral fluid was significantly lower in people with neurological diseases than in patients with lesions of other organs and body systems (p_1, p_2, $p_3<0.01$) with periodontal tissue diseases.

In order to detail the obtained results, the values of markers of the immune-inflammatory response in the oral fluid of patients with general somatic diseases with inflammatory, dystrophic-inflammatory and dystrophic diseases of periodontal tissues were analyzed.

The concentration of IL-1β in the oral fluid of subjects with inflammatory diseases of periodontal tissue significantly increased (Table I), compared to the data in the control group ($p<0.01$), but was characterized by higher values in cardiovascular patients – 72.48±4.83 ng/ml and rheumatological pathology – 81.67±5.44 ng/ml, $p_1<0.01$. At the same time, the lowest values of the level of IL-1β in oral fluid were observed in patients with neurological diseases – 58.36±3.89 ng/ml, p, $p_1<0.05$. On average, the IL-1β content in oral fluid was 1.4 times higher in patients with inflammatory diseases of periodontal tissue, respectively, compared to the data in the control group, $p<0.01$.

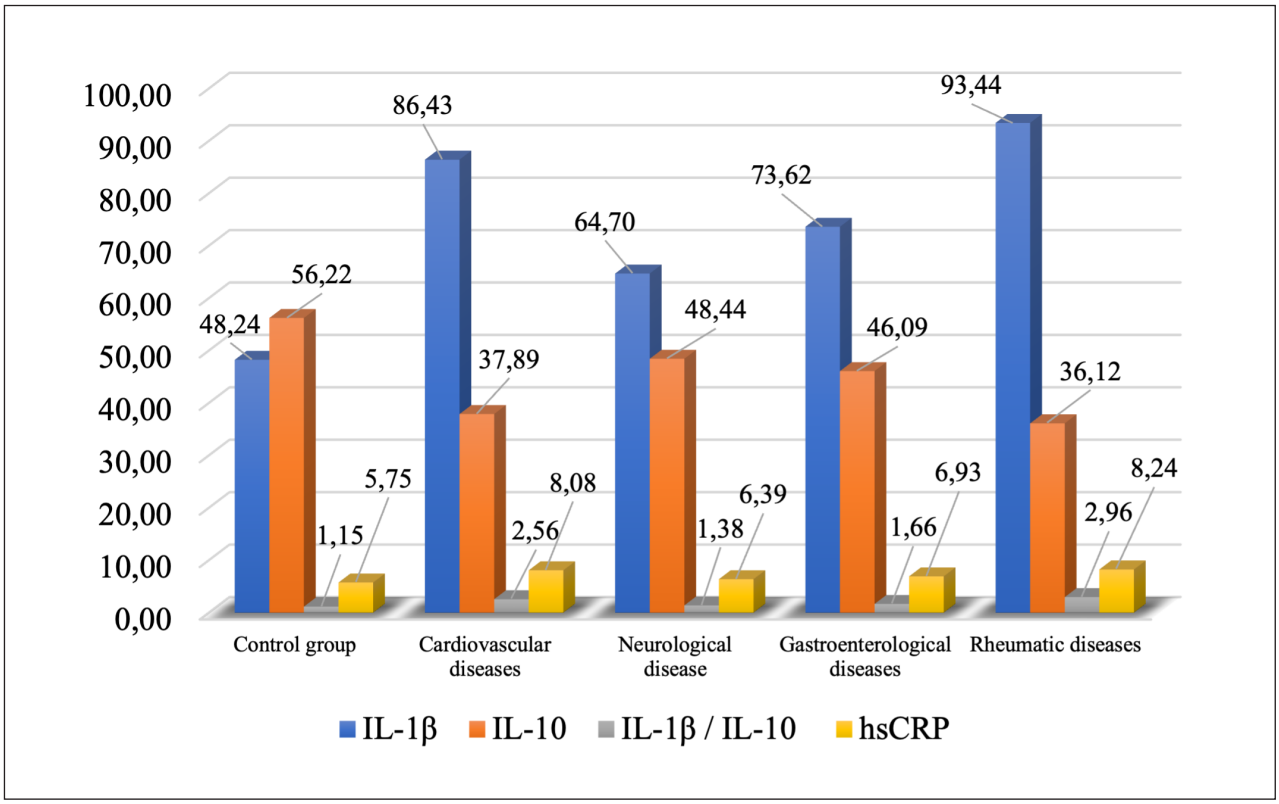

Fig. 1. The value of immune-inflammatory response markers in the oral fluid of patients with inflammatory diseases of periodontal tissues against the background of general somatic pathology

Table I. Markers of the immune-inflammatory response in the oral fluid of patients with inflammatory diseases of periodontal tissues against the background of general somatic pathology

Indicators	Cardiovascular diseases, (n=36)	Neurological diseases, (n=35)	Gastroentero-logical diseases, (n=36)	Rheumatic diseases, (n=37)	Control group, (n=30)
IL-1β, pg/ml	72,48±4,83°	58,36±3,89°°,**	65,42±4,36°	81,67±5,44°,*	48,24±3,18
IL-10, pg/ml	42,01±2,80°	50,18±3,35	48,24±3,22	40,86±2,72°,**	56,22±±3,36
IL-1β / IL-10, pg/ml	1,72±0,03°	1,16±0,09*	1,36±0,04°°,*,••	2,00±0,05°,*,•,Δ	1,15±0,08
hsCRP, mg/l	8,13±0,02°	6,20±0,04°,*	6,98±0,05°,*,•	8,29±0,06°,**,•,Δ	5,75±0,07

Notes:
°p<0,01; °°p<0,05 – a significant difference in values relative to the data in the control group.
*p_1<0,01; **p_1<0,05 – a significant difference in values in relation to data in patients with cardiovascular diseases.
•p_2<0,01; ••p_2<0,05 – significant difference of values in relation to data in patients with neurological diseases.
Δp_3<0,01 – a significant difference in values in relation to data in patients with gastroenterological diseases.

The concentration of anti-inflammatory IL-10 in the oral fluid of subjects with inflammatory diseases of periodontal tissue decreased compared to the data in the control and was characterized by certain features. Thus, the concentration of IL-10 in the oral fluid was significantly lower compared to the control only in persons with cardiovascular and rheumatological diseases, p<0.01. It was noteworthy that in patients with rheumatological pathology, the content of IL-10 in oral fluid was probably lower than in subjects with neurological diseases, p2<0.05. At the same time, on average, the level of IL-10 in oral fluid was 1.2 times lower in patients with inflammatory diseases of periodontal tissue than in controls, p<0.05.

Attention was drawn to the fact that the ratio of IL-1β / IL-10 in the oral fluid was significantly increased in patients with inflammatory diseases of periodontal tissue compared to the data in the control group in individuals with cardiovascular (p<0.01), gastroenterological (p<0.05) and rheumatological (p<0.01) diseases. On average, the ratio of cytokines in the oral fluid was 1.4 times higher in patients with inflammatory diseases of periodontal tissue compared to the data of the control group, p<0.01.

Table II. Markers of the immune-inflammatory response in the oral fluid of patients with dystrophic-inflammatory diseases of periodontal tissues against the background of general somatic pathology

Indicators	Cardiovascular diseases, (n=36)	Neurological diseases, (n=35)	Gastroentero-logical diseases, (n=36)	Rheumatic diseases, (n=37)	Control group, (n=30)
IL-1β, pg/ml	117,48±6,91°	80,54±4,74°,*	93,18±5,48°,**	120,13±7,07°,•,Δ	48,24±3,18
IL-10, pg/ml	26,49±4,42°	41,80±6,97	38,65±6,44°°	23,50±3,92°,••,ΔΔ	56,22±3,36
IL-1β / IL-10, pg/ml	4,43±0,74°	1,93±0,32°°,*	2,41±0,40°,**	5,11±0,85°,•,ΔΔ	1,15±0,08
hsCRP, mg/l	9,18±0,13°	7,25±0,11°,*	8,03±0,12°,*,•	9,34±0,14°,•,Δ	5,75±0,07

Notes:
°$p<0,01$; °°$p<0,05$ – a significant difference in values relative to the data in the control group.
*$p_1<0,01$; **$p_1<0,05$ – a significant difference in values in relation to data in patients with cardiovascular diseases.
•$p_2<0,01$; ••$p_2<0,05$ – significant difference of values in relation to data in patients with neurological diseases.
Δ$p_3<0,01$; ΔΔ$p_3<0,05$ – a significant difference in values in relation to data in patients with gastroenterological diseases.

Table III. Markers of the immune-inflammatory response in the oral fluid of patients with dystrophic diseases of periodontal tissues against the background of general somatic pathology

Indicators	Cardiovascular diseases, (n=36)	Neurological diseases, (n=35)	Gastroenterological diseases, (n=36)	Rheumatic diseases, (n=37)	Control group, (n=30)
IL-1β, pg/ml	69,33±4,22	55,21±3,68	62,27±4,15	78,52±5,23	48,24±3,18
IL-10, pg/ml	45,16±3,01	53,33±3,56	51,39±3,43	44,01±2,93	56,22±3,36
IL-1β / IL-10, pg/ml	1,53±0,05°	1,04±0,06*	1,21±0,07*	1,78±0,08°,**,•,Δ	1,15±0,08
hsCRP, mg/l	6,93±0,07°	5,73±0,05*	5,78±0,05*	7,09±0,08°,•,Δ	5,75±0,07

Notes:
°$p<0,01$ – a significant difference in values relative to the data in the control group.
*$p_1<0,01$; **$p_1<0,05$ – a significant difference in values in relation to data in patients with cardiovascular diseases.
•$p_2<0,01$ – significant difference of values in relation to data in patients with neurological diseases.
Δ$p_3<0,01$ – a significant difference in values in relation to data in patients with gastroenterological diseases.

The concentration of hsCRP in the oral fluid of the subjects was accompanied by the maximum increase in values in persons with cardiovascular ($p<0.01$) and rheumatological diseases ($p, p_2, p_3<0.01$; $p_1<0.05$). At the same time, the hsCRP content in oral fluid was higher in subjects with gastrointestinal diseases than in subjects with neurological diseases, $p_2<0.01$. On average, the hsCRP concentration in the oral fluid of individuals with inflammatory diseases of periodontal tissue of different departments exceeded the data in controls by 1.3 times, $p<0.01$.

Changes in the markers of the immune-inflammatory response in patients with dystrophic-inflammatory periodontal tissue disease against the background of general somatic pathology are presented in Table II.

It should be noted that in patients of this group, the values of the studied indicators indicated a more pronounced imbalance in the body's immunological and inflammatory response system. Thus, we determined a significant increase in the content of IL-1β in the oral fluid of the subjects with dystrophic-inflammatory periodontal tissue disease in all pathologies, $p<0.01$.

Moreover, the concentration of IL-1β in oral fluid was the lowest in individuals with neurological, $p_1<0.01$ and gastroenterological diseases, $p1<0.05$. The maximum content of IL-1β in oral fluid was determined in persons with diseases of the cardiovascular system (117.48±6.91 ng/ml, $p<0.01$) and in patients with rheumatological lesions (120.13±7.07 ng/ml, $p, p_2, p_3<0.01$). On average, the IL-1β content in the oral fluid of persons with a stroke exceeded the data of the control group by 2.1 times, $p<0.01$.

In cardiovascular, $p<0.01$, neurological, $p>0.05$, and gastroenterological pathologies, $p<0.01$, the IL-10 content in oral fluid decreased compared to control data, but did not differ in statistical significance among themselves, $p_1, p_2, p_3>0.05$. The maximum decrease of this parameter was determined in the oral fluid of patients with rheumatological lesions – 23.50±3.92 ng/ml, $p<0.01$, $p_2, p_3<0.05$. On average, the concentration of IL-10 in oral fluid in patients with dystrophic-inflammatory periodontal tissue disease was 1.7 times lower than the data in controls, $p<0.01$.

As a result of the conducted studies, a significant increase in the ratio of the content of the analyzed cytokines in the oral fluid was determined – at the minimum values of this parameter in people with neurological

diseases (1.93±0.32, p<0.05) to the maximum data in rheumatological patients (5.11 ±0.85, p<0.01), in relation to the corresponding values in the subjects of the control group. The ratio of IL-1β / IL-10 was, on average, in patients of this group in the oral fluid – 3.0 times higher compared to the data in the control, p<0.01.

The concentration of hsCRP in the oral fluid in patients with dystrophic-inflammatory periodontal tissue disease was significantly higher compared to the data in the control, p<0.01. At the same time, the maximum values of these parameters were studied in people with cardiovascular and rheumatological diseases, and the lowest values in patients with neurological lesions. However, the content of hsCRP in oral fluid in neurological and gastroenterological patients was lower than in persons with cardiovascular and rheumatological lesions, p_1, p_2, p_3<0.01. On average, the hsCRP content in the oral fluid was 1.5 times higher in patients with dystrophic-inflammatory periodontal tissue disease compared to the control data, p<0.01.

When studying changes in the markers of the immune-inflammatory response in the oral fluid of patients with dystrophic periodontal tissue diseases against the background of general somatic pathology (Table 3), it was found that the level of IL-1β in the oral fluid of patients with dystrophic periodontal tissue diseases increased by 1.4 and 1. 6 times in persons with diseases of the cardiovascular system and rheumatological lesions, respectively, p_1>0.05 and in 1.3 and 1.14 times in subjects with gastroenterological, p, p_2>0.05 and neurological diseases, p_1>0.05, respectively. On average, IL-1β content in oral fluid was 1.4 times, p>0.05 higher, compared to control data, in relation to the data in the control group.

At the same time, the lowest value of IL-10 concentration in the oral fluid was recorded in persons with dystrophic periodontal tissue diseases on the background of the cardiovascular system diseases (4.16±1.04 ng/ml, p<0.01) and with rheumatological diseases (4.00± 1.00 ng/ml, p<0.01, p_1<0.05, p_2, p_3>0.05). The level of IL-10 in the oral fluid of persons with dystrophic periodontal tissue diseases decreased, however, the obtained data did not differ statistically significantly, p_1, p_2, p_3>0.05 from the values in the control group, p>0.05. At the same time, on average, the level of IL-10 in the oral fluid was 1.2 times lower, p>0.05, in relation to the values in the control group.

The ratio of IL-1β / IL-10 in the oral fluid of patients with dystrophic periodontal tissue diseases was the highest in persons with cardiovascular system lesions (1.53±0.05, p<0.01) and in rheumatological pathology – 1.78±0, 08, p<0.01, p_1<0.05. The minimum values of the ratio of these parameters in the oral fluid were determined in patients with a neurological (1.04±0.06, p<0.01, p_1<0.01) and gastroenterological profile (1.21±0.07, p>0.05, p_1<0.01, p_2>0.05). At the same time, patients with dystrophic periodontal tissue diseases, the average value of the IL-1β / IL-10 ratio in the oral fluid was 1.2 times higher in patients in the control group, p<0.01 (Table III).

As a result of the conducted studies, an increase in the content of hsCRP in the oral fluid of persons with dystrophic diseases of periodontal tissues with general somatic pathologies was determined. At the same time, the increase in the concentration of hsCRP in oral fluid was statistically significant in patients with dystrophic diseases of periodontal tissues of all hospital departments from 5.73±0.05 mg/l in neurological patients, p>0.05, p_1<0.05 to 7.09±0.08 mg/l in persons with rheumatological diseases, p, p_2, p_3<0.01, p_1>0.05. The mean concentration of hsCRP in the oral fluid was 1.1 times higher, p>0.05, in relation to the corresponding data in the control group.

DISCUSSION

Periodontal disease has been associated with increased systemic inflammatory markers such as cytokines, which play an important role in the pathogenesis and progression of the disease, by determining the strength, nature and duration of the immune response. Cytokines in saliva have been identified as inflammatory indicators of periodontal disease and can be used as biomarkers of chronic periodontitis [16].

Interleukin 1β is considered marker of acute inflammatory phase response and it's salivary and crevicular fluid levels are strongly correlated with advanced periodontal tissue damage and periodontal infection [17, 18]. Salivary levels of this cytokine appear to serve as biomarkers in periodontitis [19]. Some previous studies showed no significant differences in salivary levels of IL-1 beta, between periodontitis patients and periodontally healthy controls and suggested that IL-1β detection in serum samples from periodontitis patients is useless for the detection of disease presence and/or its severity. Other studies suggested that concentrations of IL-1beta, were, on average, significantly higher in saliva from periodontitis patients than in healthy controls [20]. Our results regarding its saliva concentration, in patients with chronic periodontitis, show higher levels, compared to the healthy group. Moreover IL-1β concentration according to our analysis showed increasing levels along with progression of periodontal disease, showing that advanced stages of the disease may have an impact on IL1β plasmatic levels and may cause a systemic pro-inflammatory status.

IL-10 is a multifunctional anti-inflammatory cytokine with regulatory effects in periodontal inflammation. It has beneficial effects in limiting the excessive immune response, tissue destruction and bone resorption in periodontal disease, by inhibiting the synthesis of pro-inflammatory cytokines [21, 22]. It has been reported that lower amounts of IL-10 may contribute to the progression of periodontal disease and that higher amounts have protecting effect on tissue destruction, by suppressing the secretion of pro-inflammatory cytokines, such as IL-1 [21,22]. In saliva, we found higher concentrations of IL-10 in healthy patients compared to patients with different stages of periodontal disease. It should be noted, that the maximum decreasing levels of this interleukin in patients with the dystrophic-inflammatory periodontal tissue diseases. Other studies also found negative correlations between clinical parameters of periodontal disease and saliva levels of IL-10 [23]. Also saliva levels of IL-10 were found to be low in periodontitis and metabolic syndrome patients and periodontal treatment showed improvement of saliva levels of this interleukin [24]. However periodontitis and non-periodontitis subjects may present a similar ability to produce this regulatory cytokine and the exact relationship amongst periodontal diseases, systemic inflammation and cytokines still remains unclear [25].

CRP is an acute-phase protein mainly produced by the liver inresponse to infection or tissue damage [26, 27]. While CRP is amore traditional inflammation marker with less precision(within the range of 10 to 1,000 mg/L), high sensitivity CRP (hs-CRP) is a highly precise and accurate analyte (within therange of 0.5 to 10 mg/L) [28]. The association between CRP andperiodontitis received great attention in part due to the linkbetween periodontitis and cardiovascular disease [29]. However, CRP has been established as a marker of associationof periodontitis with other systemic diseases [30]. It should be noted that in our study, an increase in the level of hs CRP in oral fluid was also observed in patients with periodontal tissue diseases against the background of general somatic pathology. Previous systematic reviews suggested elevated CRP levels inpatients with periodontitis [31-33].

Recent evidence has indicated that patients with periodontitis have increased saliva levels of CRP, when compared with unaffected control population [34]. But they fall short in indicating that periodontitis was the cause for the observed saliva CRP levels as CRP levels fluctuate with various factors like: aging, somatic diseases, high blood pressure, alcohol use, smoking, low levels of physical activity, chronic fatigue, coffee consumption having elevated triglycerides, insulin-resistance diabetes, taking estrogen, eating a high-protein diet, depression [35].

Various studies have proved a positive association between the presence of chronic periodontitis and high saliva CRP levels [36-38] because it is biologically plausible that inflammatory mediators (IL-1, IL-6 and TNF-α) are released under conditions of periodontitis and present the capacity to stimulate the hepatocytes to produce CRP.

Our results suggest, that in patients with general somatic pathology, elevated concentrations of IL-1β, IL-1β / IL-10, hsCRP and decrease IL-10 in oral fluid, closely associated with condition of periodontal tissues. The correlation between clinical periodontal disease and oral fluid concentrations of these cytokines, observed in our study, suggests that a causal relationship between diseases of periodontal tissues and systemic inflammation might exist.

CONCLUSIONS

So, we found that, on average, the concentration of IL-1β – 1.5 times, IL-1β/IL-10 – 1.4 times, hsCRP – 1.3 times increased in the oral fluid of patients with inflammatory periodontal diseases, $p<0.01$, compared to the values in the control group. In the patients main group with dystrophic-inflammatory diseases of periodontal tissues, the content of IL-1β, IL-1β/IL-10 and hsCRP in the oral fluid was 2.1 times, 3.0 times and 1.5 times higher, $p<0.01$, whereas, the IL-10 content was 1.7 times lower, $p<0.01$, compared to the indicators in the control group. On average, in patients with dystrophic periodontal diseases, the content of IL-1β in the oral fluid was 1.4 times, IL-1β/IL-10 – 1.2 times, hsCRP – 3.1 times, $p<0,01$, higher, whereas, the content of IL-10 was 1.2 times lower, $p>0.05$, in relation to the values in the control group.

Therefore, in patients with diseases of periodontal tissues in combination with general somatic pathology, a probable increase in the level of the pro-inflammatory cytokine IL-1β and C-reactive protein was determined, against the background of a decrease in the anti-inflammatory cytokine IL-10 in the oral fluid, compared to the values in the control group.

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ORIGINAL ARTICLE

THE IMPORTANCE OF THE PROGNOSTIC SCORE FOR THE CHOICE OF CHIVA HEMODYNAMIC SURGERY AS A TREATMENT METHOD FOR VARICOSE VEINS OF THE LOWER EXTREMITIES

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ABSTRACT

The aim: To work out the predictive system that can help to determine the group of patients to whom the hemodynamic surgery of varicose disease, CHIVA, is beneficial.

Materials and methods: Results of examination and treatment of 58 patients of the main group who underwent hemodynamic surgery and 65 patients of the comparison group who underwent stripping. Patients of both groups were evaluated in the preoperative period using an evaluation scale, and divided into three subgroups depending on the scores: 5- 8, 9 – 11, and 12 – 15 points.

Results: The best treatment results with the lowest number of relapses were obtained in the subgroup of patients with low scores on the prognostic scale (5-8 points) after hemodynamic treatment and in the subgroup of patients with a high the number of points (12 -15 points) after the classic stripping ($p < 0.05$). The same subgroups received more improvement in the quality of life of patients according to CIVIQ 20 ($p < 0.001$). The subgroup of patients with a high number of points (12 -15 points) after the stripping received significantly more reduction in scores VCSS ($p < 0,01$).

Conclusions: Comprehensive assessment of factors such as the anamnestic duration of the disease, the diameter of the great saphenous vein, the presence of skin complications, dilated varicose collaterals and previous surgical treatment using a prognostic preoperative assessment score allows the surgeon to be more clearly guided in choosing the optimal method of treatment for each patient and achieve the best treatment results.

KEY WORDS: varicose veins, stripping, hemodynamic surgery, CHIVA

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INTRODUCTION

CHIVA (Cure Conservatrice et Hemodynamique de l'Insufficience Veineuse en Ambulatoire) is a type of mini-invasive surgery for varicose vein disease that avoids destroying the saphenous vein and its collaterals [1].

Based on a theoretical hemodynamic model, CHIVA is an ultrasound-guided, minimally invasive surgical strategy performed under local anesthesia. The strategy has been shown in studies to be safe and effective [2].

CHIVA is a good alternative to common procedures that is associated with less bruising, nerve damage than stripping saphenectomy. The main advantages are preservation of the saphenous vein, local anesthesia, low cost, low pain, and fast post-operative recovery [3].

Despite the many advantages of the hemodynamic approach of CHIVA, there are some disadvantages of this technique. The main disadvantages for the patient are the relatively high frequency of recurrences, and prolonged onset of clinical and cosmetic effects. For doctors, the main disadvantage is the need to train in features of venous hemodynamics and ultrasound mapping [3, 4].

Taking into account all these facts, an important question arises: which category of patients is best suited to the hemodynamic approach of CHIVA, for whom it will be most effective and efficient, and which category of patients with varicose veins is better to choose other treatments.

THE AIM

The purpose of the work is to work out the predictive system that can help to determine the group of patients to whom the hemodynamic surgery of varicose disease, CHIVA, is beneficial.

MATERIALS AND METHODS

The results of the examination and hemodynamic treatment of 58 patients with varicose veins of the lower extremities and symptoms of chronic venous insufficiency (CVI), who were hospitalized in the clinic in the period from 2018 to 2021 were analyzed. The comparison group consisted of 65 patients with varicose veins of the lower extremities who underwent stripping.

The inclusion criteria:

1) presence of varicose veins (C1 – C6 class of varicose veins according to CEAP classification);
2) age from 18 to 65 years;
3) saphenofemoral junction insufficiency;
4) good drainage in the deep venous system;
5) consent of the patient to participate in the study.

Exclusion criteria:

1) patients with severe comorbidities that may affect the course and outcome of treatment (diabetes mellitus, autoimmune diseases, oncological diseases, kidney failure, liver failure, heart failure, severe lung diseases);
2) deep venous system obstruction.
3) lack of compliance with the use of compression therapy and its duration.
4) patient disagreement.

In addition to general clinical examinations, all patients were required to undergo ultrasound duplex scanning (UDS) veins of the lower extremities in the supine and standing positions, using the of Paraná manoeuvre and Valsalva tests, with detailed mapping of the venous hemodynamics of the lower extremities. Ultrasound examination was performed according to a standardized scheme [5-7].

Based on the researchers' data and our own results, we selected the criteria that most influenced or could influence the outcome of hemodynamic treatment. The following criteria we included:

- duration of the disease – time limits, which we divided into: up to 5 years, 5-10 years, and more than 10 years (according to existing data, prolonged overstretching of venous structures by high pressure leads to the fact that when the pressure normalizes, the latter on the background of existing sclerotic processes cannot restore its original form);
- diameter of the great saphenous vein (GSV) according to ultrasound scanning;
- skin complications: pigmentation, venous eczema, lipodermatosclerosis, healed ulceration, active ulceration;
- visible extended tributaries (multiple varicose collaterals, tributaries, and nodules that can cause unsatisfactory cosmetic treatment results for the patient);
- previous surgical intervention in the anamnesis (especially the interventions performed on the GSV. Surgical treatment, first, strongly changes the hemodynamics. In such patients, it is difficult to determine the type of shunt, and therefore the right strategy. Secondly, if we are already dealing with relapse, it may indicate a high ability of veins to recanalize).

We grouped the above data as a scale, giving each of the parameters the appropriate number of points (Table I).

We evaluated all our patients according to the proposed scale and divided them into subgroups according to the obtained data. The first subgroup (A) included patients who scored from 5 to 8 points – their number was 22, the second subgroup (B) included 19 patients who scored from 9 to 11 points, and the third subgroup (C) included patients who scored the highest number of points – from 12 to 15 and the number of such patients was 17.

Patients in the comparison group were also evaluated according to our proposed scale and divided into appropriate subgroups: 5-8 points (A1) – 26 patients, 9-11 (B1) – 23, 12-15 points – 16 patients (C1), respectively.

Table I. Prognostic score of preoperative assessment of patients with varicose veins of the lower extremities

	Attribute	1 point	2 points	3 points
1	Duration of the disease	Up to 5 years	5-10 years	More than 10 years
2	The diameter of the GSV according to UDS	Up to 7.5 mm	7.5 -10 mm	More than 10 mm
3	Skin complications	None	Pigmentation, venous eczema, lipodermatosclerosis	Healed ulceration or active ulceration
4	Visible tributaries	None or reticular varicose veins and telangiectasias	Several: 1-3 veins up to 0.5 cm in diameter	Multiple nodes, varicose tributaries and collaterals
5	History of previous surgical treatment	No	Sclerotherapy, miniphlebectomy of tributaries, without intervention on GSV	Safenectomy, or any intervention on GSV

Table II. Clinical results of treatment

Evaluation period - 6 months												
Treatment result	Groups of patients											
	Main group						Comparison group					
	A (Total =22)		B (Total =19)		C (Total =17)		A1 (Total =26)		B1 (Total =23)		C1 (Total =16)	
	n	%	n	%	n	%	n	%	n	%	n	%
Complete recovery	14	63,6	9	47,4	5	29,4	13	50	13	56,5	9	56,2
Improvement	6	27,3	7	36,8	4	23,5	10	38,5	9	39,2	5	31,3
Absence of positive changes	2	9,1	3	15,8	8	47,1	3	11,2	1	4,3	2	12,5
Evaluation period - 12 months												
Treatment result	Groups of patients											
	Main group						Comparison group					
	A (Total =22)		B (Total =19)		C (Total =17)		A1 (Total =26)		B1 (Total =23)		C1 (Total =16)	
	n	%	n	%	n	%	n	%	n	%	n	%
Complete recovery	15	68,2	9	47,4	5	29,5	12	46,2	10	43,5	9	56,3
Improvement	5	22,7	8	42,1	3	17,6	11	43,3	10	43,5	5	31,2
Absence of positive changes	2	9,1	2	10,5	9	52,9	3	11,5	3	13	2	12,5

Table III. Treatment results according to VCSS

Assessment time	Groups of patients					
	Main group			Comparison group		
	A (N=22)	B (N=19)	C (N=17)	A1 (N=26)	B1 (N=23)	C1 (N=16)
Before treatment	3,86±0,41	9,26±0,82	16,44±1,84	3,58±0,39	9,57±0,69	15,5±1,88
6 months after treatment	1,59±0,4*	3,11±0,9*	6,94±1,55*	2,23±1,53*	2,13±0,31*	2,19±0,33*
12 months after treatment	1,68±0,48*	3±0,81*	6,81±1,25*	2,27±1,54*	2,3±0,38*	2,34±1,36*

*p<0,01 Multiple comparisons. Dunn test, compared with the before treatment assessment.

Table IV. The results of treatment according to the CIVIQ 20, presented in the GIS index presented in the GIS index

Assessment time	Groups of patients					
	Main group			Comparison group		
	A (N=22)	B (N=19)	C (N=17)	A1 (N=26)	B1 (N=23)	C1 (N=16)
Before treatment	76,77±2,44	69,37±1,96	34,18±2,86	75,92±2,24	68,17±1,72	34,25±3,05
6 months after treatment	98,09±0,96*	92,47±1,92*	76,59±2,63*	92,77±0,98*	94,13±1,55*	90,63±2,07*
12 months after treatment	98,27±0,97*	93±1,79*	75,53±2,52*	92,08±1,04*	92,74±1,86*	90,25±2,15*

*p<0,01 (Multiple comparisons. Dunn test), compared with the before treatment assessment.

Patients in the main group underwent hemodynamic treatment according to the principles of CHIVA. Patients in the comparison group underwent stripping, supplemented by miniphlebectomy and ligation of perforating veins.

The results of treatment were classified into three groups:

- complete recovery (complete absence of varicose veins and symptoms of CVI);
- improvement (visible residual or newly formed varicose veins or nodes that have no or little clinical and

hemodynamic significance);
- absence of positive changes (complete recurrence of varicose veins, recurrence of symptoms of CVI).

Separately, we counted the number of relapses.

Conducted a score using Venous Clinical Severity Scoring (VCSS) (according to Clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum) [8].

Satisfaction with the results of treatment was also assessed using a Chronic Venous Insufficiency quality of life Questionnaire (CIVIQ 20), in order to compare the average scores, the absolute scores were converted to a GIS index [9].

Treatment outcomes were assessed 6 and 12 months after treatment.

Based on the results, the significance of the proposed criteria for determining the group of patients for whom hemodynamic surgery would be the best choice was evaluated.

Statistical analysis was performed using Statistica 10 (Serial Number: STA999K347150-W) and MedStat. Data distribution normality was checked using the Shapiro-Uilk criterion. A comparison of the data between the groups was performed using Wilcoxon two-sample test. Multiple comparisons were performed using Rank Kruskal-Wallis test and Dunn's test, Scheeffe"s method for multiple comparisons.

RESULTS

• **Complete recovery (complete absence of varicose veins and symptoms of CVI).**

In the main group in subgroup A after 6 months, 14 patients fully recovered (63,6%), subgroup B – 9 (47,4%), subgroup C – 5 (29,4%) In the comparison group (patients underwent stripping + miniphlebectomy + elimination of perforant) complete recovery was achieved in 13 (50%) patients of subgroup A1, 13 (56,5%) – subgroup B1 and 9 (56,2%) – subgroup C1. After a year of follow-up, the results were: subgroup A – 15 patients (68,2%), B – 9 (47,4%), C – 5 (29,5%); subgroup A1 – 12 (46,2%), B1 – 10 (43,5%) C1 – 9 (56,3%) persons accordingly.

• **Improvement (visible residual or newly formed varicose veins or nodes that have no or little clinical and hemodynamic significance);**

In the main group in subgroup A after 6 months insignificant residual veins were observed in 6 patients (27,3%), subgroup B – 7 (36,8%), subgroup C – 4 (23,5%). In the comparison group, improvements were achieved in 10 (38,5%) patients of the subgroup A1, 9 (39,2%) – subgroup B1 and 5 (31,3%) – subgroup C1. After one year of follow-up, the results were: subgroup A – 5 patients (22,7%), B – 8 (42,1%), C – 3 (17,6%); subgroup A1 – 11 (43,3%), B1 – 10 (43,5%) C1 – 5 (31,2%) persons accordingly.

• **Absence of positive changes (complete recurrence of varicose veins, recurrence of symptoms of CVI).**

In the main group in subgroup A after 6 months recurrence of varicose veins, return of symptoms of CVI was observed in 2 patients (9,1 %), subgroup B – 3 (15,8%), subgroup C – 8 (47,1%). In the comparison group, no clinical changes were achieved in 3 (11,2%) patients of the subgroup A1, 1 (4,3%) – subgroup B1 and 2 (12,5%) – subgroup C1. After one year of follow-up, the results were: subgroup A – 2 patients (9,1%), B – 2 (10,5%), C – 9 (52,9%); subgroup A1 – 3 (11,5%), B1 – 3 (13%), C1 – 2 (12,5%) persons accordingly.

The results are grouped and shown in table (Table II).

There was no statistically significant difference between the main group and the comparison group when comparing subgroups, A and A1 ($p = 0.266$), between subgroups B and B1 ($p = 0.334$), but between subgroups C and C1 found a statistically significant difference at the level of significance ($p < 0.05$). After one year of follow-up, the data changed slightly, but this did not lead to statistically significant changes in the results $p > 0.05$. We would like to note that in the main group there was a trend in subgroups A and B to improve treatment outcomes in the assessment period of one year, compared to the 6-month period, and in subgroup C1 from the comparison group the most stable result among all subgroups during the whole observation period was noted.

The number of relapses in different subgroups was separately assessed and compared. The highest number of relapses was registered in subgroup C of the main group – 8 in total, which is statistically significant in comparison to subgroups A and B of the main group. ($p < 0,05$, Rank Kruskal-Wallis test.), and in comparison with the corresponding subgroup of the comparison group C1 ($p = 0,035$, Wilcoxon two sample test).

Evaluation of treatment results on the VCSS scale are given in table (Table III).

As shown in the table, the VCSS score in subgroups A was 3.86±0.41, B – 9.26±0.82, C – 16.44±1.84, A1 was 3.58±0.39, B1 – 9.57±0.69, and C1 15.5±1.88, respectively. The difference in score between subgroups A and C, and A1 and C1 before treatment was more than 75%. One year after treatment, the scores in subgroups A was 1.68±0.48, B – 93±0.81, C – 6.81±1.25, A1 – 2.27±1.54, B1 – 2.3 ±0.38, and C1 – 2.34±1.36, respectively. The difference between subgroups A and C was still about 70%, while the difference in VCSS between A1 and C1 after treatment was about 3% ($p < 0.01$).

Analyzing the data in Table 2, it can be stated that patients of all subgroups showed a statistically significant decrease in VCSS after treatment at an assessment time of 6 months and one year ($p < 0,01$). In the one-year period, compared to 6 months, there was a slight increase in all groups, but not statistically significant. The strongest decrease was observed in subgroups A, which underwent hemodynamic treatment and subgroup C1 (stripping). There was a statistically significant difference in the decrease in VCSS between similar subgroups C and C1 of the main group and the comparison group in the 6-month period ($p = 0.006$) and the annual period ($p = 0.008$), according to which subgroup C1 received significantly more reduction in VCSS after treatment in both terms of assessment.

The obtained results suggest that a more stable result is suitable for both patients with a low score on the prognostic preoperative scale and high scores can be obtained with stripping, while hemodynamic surgery is more suitable for patients with a low score.

The questionnaire CIVIQ 20 was also used to evaluate treatment outcomes. Relevant data are shown in table (Table IV).

GIS index was in subgroups A at the level 76,77±2,44, B – 69,37±1,96, C – 34,18±2,86, A1 was 75,92±2,24, B1 – 68,17±1,72, and C1 34,25±3,05. The value of the index for subgroups A and A1 was determined to a greater extent by subjective symptoms related to cosmetic dissatisfaction and psychological discomfort, while for subgroups C and C1 physical symptoms of chronic venous insufficiency were in the first place.

Based on the results shown in the table, in all groups there was a statistically significant increase in the quality of life of patients after treatment for all subgroups ($p < 0.01$). It should be noted that in subgroup A there was a greater increase in the GIS index compared to subgroup A1 ($p < 0.001$), and in subgroup C1, on the contrary, the GIS index was higher compared to subgroup C ($p < 0.001$). There was no statistically significant difference in subgroups B and B1 ($p = 0.739$).

We proposed to use this scale to 15 colleagues from 4 surgical departments in Ukraine who treat varicose veins of the lower extremities and practice the hemodynamic approach CHIVA. A year later, we asked colleagues about their experience of using our scale and its usefulness. Among the respondents 93.3% said that the scale is simple and easy to understand, 86.6% used it in their daily routine. 73.3% of respondents noted its usefulness in the clinical evaluation of patients with varicose veins of the lower extremities and the choice of treatment. More than half of the respondents – 53.3% said that the result obtained according to the scale coincides with their opinion on the choice of treatment, and 26.6% relied entirely on the scale when choosing a method of treatment. And 86.6% of respondents confirmed that they will continue to use the scale in their daily practice.

DISCUSSION

Varicose vein surgery is being increasingly offered and has many techniques in its arsenal. Much attention is now being paid to minimally invasive and hemodynamic techniques, but classic techniques such as stripping continue to be widely used. Each technique shows a number of advantages, but a number of disadvantages are also described in some groups of patients [10, 11] The results of treatment, the presence of relapses, as well as the occurrence of varicose veins itself, are influenced by many different factors. The presence of trophic ulcers as factors affecting the results of treatment are distinguished by many scientists. The influence of chronically increased intra-abdominal pressure due to obesity, and a history of deep venous thrombosis are also noted [12].

There are also a number of factors that can affect the outcome of the operation and the course of the postoperative period, both in vein surgery and in other surgical interventions, for example in abdominal surgery. Such factors include age, concomitant diseases, features of hemocoagulation, constant intake of hormonal drugs and some other medications, etc [13-15]. These factors are sometimes difficult to identify and take into account their influence on the effect of treatment.

Use of the CEAP classification system is important for diagnosis but does not provide guidance for treatment decisions [16]. For this topical issue for all surgeons is the choice of a specific technique for a particular patient.

Based on our experience in treating patients with varicose veins and literature data, we have identified several factors that could affect the results of operations to a greater extent. The results show that factors such as the duration of the disease, the diameter of the GSV, the presence of skin complications, visible varicose veins and previous history of surgical treatment affect the outcome of treatment, therefore, a comprehensive assessment of these factors using a scale allows the surgeon to better navigate and choose a method of treatment.

For patients with a short-term disease history, small GSV diameter, no or minor skin complications, no visible varicose tributaries and collaterals, and a history of previous surgical treatment, hemodynamic treatment will be the best option. This is clearly demonstrated by the assessment with the CIVIQ 20 questionnaire, which

combines not only an objective scale but also a subjective one, primarily related to the cosmetic comfort of the patient.

For patients with long-term disease history, large vein diameter, skin complications, numerous varicose tributaries and collaterals, the presence of trophic ulcers, para ulcer eczema, and surgical treatment in the anamnesis, the best choice will be stripping with careful elimination of pathological perforators and communicators and miniphlebectomy. For this category of patients, it will provide the most stable functional result with the minimum number of relapses and will give a satisfactory cosmetic treatment result.

CONCLUSIONS

1. The prognostic scale allowed to achieve the optimal surgical approach in 68.2% of patients with a low score (5-8 points) and this was hemodynamic treatment according to the principles of CHIVA and in 56.3% of patients with high scores (12-15 points) when choosing a treatment method stripping, supplemented by miniphlebectomy and ligation of perforating veins ($p < 0.05$).

2. Highest relapse rate, compared to other subgroups, was observed in the subgroup of patients with high scores on the prognostic preoperative scale (12 -15 points) after hemodynamic treatment ($p < 0,05$).

3. The highest level of improvement in the quality of life of patients according to CIVIQ 20 was obtained in the subgroup of patients with low scores on the prognostic preoperative scale (5-8 points) after hemodynamic treatment, and in the subgroup of patients with high the number of points (12 -15 points) after the classic stripping supplemented by miniphlebectomy and elimination of perforators ($p < 0.001$).

4. The prognostic preoperative assessment scale is a simple, easy-to-understand, and accessible tool that can be used in the daily routine practice of a phlebologist. Nearly 90% of respondents gave positive feedback after using the proposed scale.

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ORIGINAL ARTICLE

PERIODONTAL DISEASE AND SALIVARY OXIDATION STRESS IN CHILDREN WITH LYMPHOGRANULOMATOSIS

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ABSTRACT

The aim: To investigate the impact of lymphogranulomatosis (LGM) and periodontal disease on salivary lipid peroxidation and enzymatic antioxidants` levels in children.

Materials and methods: 45 children aged 6–16 years with LGM were examined before hematologic therapy (group LGM 1), after therapy (group LGM 2), and at the remission (group LGM 3). The control group included 70 healthy children. Periodontal state of children, saliva thiobarbituric acid reacting substances (TBARS), superoxide dismutase (SOD) and catalase were examined.

Results: 6-11 years old children from LGM 1 group showed a higher frequency of periodontal disease (50,0%), as well as 12-15 year olds (80,8%) compared to healthy children (17,4% and 42,8% accordingly, $p<0,05$). TBARS levels were higher in LGM 1-3 groups of children with periodontal disease (9,79, 12,3 and 12,6 umol/l, $p<0,01$) compared to counterparts without it (8,01, 10,1 and 11,6 umol/l, $p<0,01$) and healthy children with periodontal disease (7,9 umol/l, $p<0,01$). SOD activity was higher in LGM 1-3 groups of children with periodontal disease (-0,075, -0,086, -0,074 units) compared to children without it (-0,048, -0,059, -0,04 units, $p<0,01$) and healthy children with periodontal disease (-0,04 units, $p<0,01$). Catalase activity was lower in LGM 1-3 groups of children with periodontal disease (6,72, 5,2 and 6,7 units) compared to counterparts without it (7,3, 3,7 and 4,7 units, $p<0,01$) and healthy children with periodontal disease (7,1 units, $p<0,01$).

Conclusions: Children with periodontal disease related to LGM had higher TBARS levels, SOD activity and lower catalase activity in saliva. Both LGM and periodontal disease altered lipid peroxidation and antioxidant protection in saliva of children.

KEY WORDS: children, periodontal disease, oxidative stress, antioxidant, lymphogranulomatosis

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INTRODUCTION

Lympogranulomatosis (LGM) or Hodgkin`s lymphoma is considered as a highly curable blood malignant disease with distinct clinical, histological, and biologic manifestations [1,2]. One of the pathogenetic mechanisms of cancerogenesis involves high levels of reactive oxygen species because of metabolic and signaling abnormalities [3]. Oxidation stress develops as a result of imbalance between lipoperoxidation and antioxidant system. Superoxide anion radicals cause lipoperoxidation in the plasma membrane of surrounding host cells leading to the production of hydroxynonenal and malondialdehyde, the basic components of thiobarbituric acid reacting substances (TBARS). The antioxidant system contains both enzymatic and non-enzymatic antioxidants, and the main enzymes are superoxide dismutase (SOD) and catalase.

It has been shown that the patients with non-Hodgkin`s lymphoma at the permission phase had a higher level of reactive oxygen metabolites and a lower antioxidant potential in serum as compared to healthy volunteers [4].

Children with blood malignancies have different oral manifestations, but only the study of Simon et al. [5] dealt with this periodontal disease related to LGM. Moreover, many studies have been conducted to identify the correlation between oxidative stress and periodontal disease, in which the markers of oxidative stress are examined in saliva, blood and gingivae. The non-invasive sampling makes saliva particularly useful in the research on children, especially with oral manifestations of malignant diseases. Hendi et al. [6] showed that the periodontal disease-active patients had higher catalase and lower superoxide dismutase activity as compared to the periodontal disease-free counterparts. The total antioxidant capacity level in saliva increased in children aged 3-18 years with higher gingival bleeding [7]. However, Ghallab et al. [8] demonstrated superoxide dismutase decrease in gingival crevicular fluid of aggressive and chronic periodontitis patients. In addition,

Tóthová et al. [9] claimed that salivary TBARS related to papillary bleeding index in children.

The authors hypothesized that both LGM and periodontal disease have the impact on salivary lipid peroxidation and enzymatic antioxidants` levels in children.

THE AIM

To investigate the impact of LGM and periodontal disease on salivary lipid peroxidation and enzymatic antioxidants` levels in children.

MATERIALS AND METHODS

45 children aged 6–16 years who had been diagnosed with LGM and underwent hematological treatment in the Pediatric Hematology Ward of Children Hospital (Poltava, Ukraine) were included in this study. This cross-sectional study was carried out in 2013-2019 in Poltava, Ukraine in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Bioethical Committee of PDMU, and parents received a complete explanation about the aims and methods of the study, and signed written consent. Patients were treated according to international protocols of chemotherapy or had radiotherapy.

Patients with LGM were categorized into three groups. Group LGM 1 – patients examined before the hematologic therapy; group LGM 2 – patients examined 1 month after the therapy; group LGM 3 – patients examined while in permanent remission (1-5 years). The control group included 70 healthy children aged 6–16 years without a history of a systemic disease.

Methods of this study were periodontal examination and screening of salivary lipid peroxidation and enzymatic antioxidants. Periodontal examination was performed in compliance with the guidelines for periodontal screening in children [10]; the complaints, medical history, and clinical criteria (gum color, swelling, bleeding during periodontal probing) were used to make diagnosis of gingivitis or periodontitis in examined children. A periodontal probe was utilised for examination (AEP WHO B; Hu-Friedy). Frequency of gingivitis and periodontitis was evaluated. The children were instructed to clean teeth with a soft brush or a cotton pick.

Saliva samples were collected from children similar to this previously reported Vahabzadeh et al. [11]. The samples were stored frozen at −20°C and were processed as soon as possible to minimize the effects of storage. Clinical unstimulated salivary samples were collected from 40 healthy children and 25 children with LGM. Lipid oxidation was assessed by increasing in the concentration of TBARS during 1,5 hours incubation in iron-ascorbate buffer solution. TBARS was analyzed in the samples by the formation of a stained trimethine complex during the reaction of tiobarbituric acid [12]. Also the activity of antioxidant enzymes – SOD and catalase was studied [12].

The Chi-square test was used to explain the significant differences in periodontal disease frequencies. Categorical variables of salivary indices were analyzed by Student's t-test. Statistical significance level of $p<0,05$ was applied for all calculations.

RESULTS

The children from LGM 1 group showed a significantly higher frequency of periodontal disease as compared with the control group in 6-11 year olds (50,0% vs 17,4%) and 12-15 year olds (80,8% vs 42,8%, $p<0,05$) (Table I). Children from LGM 2 group showed a higher frequency of periodontal disease in 6-11 year olds (60%) and 12-15 year olds (90%) compared with the control group ($p<0,05$) (Table II). Same trend was found in the LGM 3 group (Table III). The frequency of periodontal disease increased in 6-11 years old children over the course of LGM ($p<0,05$). Mostly, gingivitis represented periodontal disease and only 12-16 year olds from LGM 3 group had periodontitis at 16% cases.

Healthy children with periodontal disease had higher TBARS level than counterparts without it (7,9 vs 5,7 umol/l, $p<0,001$). TBARS level in LGM 1 group without periodontal disease (8,01 umol/l) was higher than in healthy children ($p<0,01$) (Table II). This index increased in the LGM 1 group with periodontal disease (9,79 umol/l) as compared to the control group with periodontal disease ($p<0,001$) and LGM 1 group without periodontal disease ($p<0,01$). TBARS level increased over the course of LGM, and was higher in the LGM 2 group with periodontal disease (12,3 umol/l) as compared to the healthy children with periodontal disease ($p<0,001$) and LGM 2 group without periodontal disease (10,1 umol/l, $p<0,01$) (Table III). Similar trend was found in the children from LGM 3 group (Table IV), moreover this index was higher in children without periodontal disease (11,6 umol/l) and with periodontal disease (12,6 umol/l) as compared to the LGM 2 group ($p<0,01$).

Healthy children with periodontal disease had higher SOD activity than counterparts without it (-0,04 vs -0,02 units, $p<0,01$). SOD activity in LGM 1 group without periodontal disease (-0,048 units) was higher than in healthy children ($p<0,01$) (Table II). This index increased in LGM 1 group with periodontal disease (-0,075 units) as compared to the control group with periodontal disease ($p<0,01$) and LGM 1 group without periodontal disease

Table I. Frequency of periodontal disease in children 6-16 years with LGM

Group	Age, years	n	Frequency of periodontal disease	
			n	%
Control	6-11	35	12	17,4±6,1
LGM 1	6-11	20	10	50,0±11,1
				p_1<0,01
LGM 2	6-11	20	12	60,0±10,9
				p_1<0,01 p_2>0,05
LGM 3	6-11	20	16	80,0±8,9
				p_1<0,01 p_2<0,05
Control	12-16	35	15	42,8±6,2
LGM 1	12-16	20	16	80,8±8,9
				p_1<0,01
LGM 2	12-16	20	18	90,0±6,7
				p_1<0,01 p_2>0,05
LGM 3	12-16	25	19	76,0±8,7
				p_1<0,01 p_2>0,05

Note: p_1 – as compared to control group; p_2 – as compared to LGM 1 group

Table II. Lipid perioxidation and enzymatic activity in saliva of LGM 1 group

Index	LGM 1 group, n=20		Control group, n=40	
	Healthy periodontium, n=7	Periodontal disease, n=13	Healthy periodontium, n=25	Periodontal disease, n=15
TBARS umol/l	8,01±0,31	9,79±0,18	5,70±0,18	7,90±0,55
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		p_1<0,001
SOD, activity units	-0,048±0,0045	-0,075±0,0063	-0,02±0,007	-0,04±0,0038
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		p_1<0,001
Catalase, activity units	7,30±0,35	6,72±0,55	8,97±0,24	7,10±0,32
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		p_1<0,001

Note: p_1 – as compared to control group with healthy periodontium, p_2 – as compared to control group with periodontal disease, p_3 – as compared to LGM group with periodontal disease

(p<0,001). SOD activity was higher in the LGM 2 group with periodontal disease (-0,086 units) as compared to the healthy children with periodontal disease (p<0,001) and the LGM 2 group without periodontal disease (-0,059 units, p<0,01) (Table III). Similar trend was found in the children from LGM 3 group (Table IV), however, SOD activity decreased in LGM children without periodontal disease (-0,04 units) and with it (-0,074 units) as compared to parameters of the LGM 2 group (p<0,01).

Healthy children with periodontal disease had lower catalase activity than counterparts without it (7,1 vs 8,97 units, p<0,001). Catalase activity in LGM 1 group without periodontal disease (7,3 units) was lower than in healthy children (p<0,001) (Table II). This index decreased in LGM 1 group with periodontal disease (6,72 units) as compared to the control group with periodontal disease (p<0,01) and LGM 1 group without periodontal disease (p<0,01). Catalase activity was

Table III. Lipid perioxidation and enzymatic activity in saliva of LGM 2 group

Indices	LGM 2 group, n=20		Control group, n=40	
	Healthy periodontium, n=7	Periodontal disease, n=13	Healthy periodontium, n=25	Periodontal disease, n=15
TBARS, umol/l	10,10±0,29	12,30±0,24	5,70±0,18	7,90±0,55
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		
SOD, activity units	-0,059±0,0046	-0,086±0,0078	-0,02±0,002	-0,04±0,0038
	p_1<0,01 p_2<0,01 p_3<0,01	p_1<0,01 p_2<0,001		
Catalase, activity units	5,10±0,45	3,70±0,65	8,97±0,24	7,10±0,32
	p_1<0,01 p_2<0,01 p_3<0,01	p_1< 0,01 p_2 < 0,001		

Note: p_1 – as compared to control group with healthy periodontium, p_2 – as compared to control group with periodontal disease, p_3 – as compared to LGM group with periodontal disease

Table IV. Lipid perioxidation and enzymatic activity in saliva of LGM 3 group

Index	LGM 3 group, n=25		Control group, n=40	
	Healthy periodontium, n=7	Periodontal disease, n=13	Healthy periodontium, n=25	Periodontal disease, n=15
TBARS, umol/l	11,60±0,92 p_1<0,01 p_2<0,01 p_3<0,01 p_4<0,01	12,60±1,21 p_1<0,01 p_2<0,001 p_4<0,01	5,70±0,18	7,90±0,55
SOD, activity units	-0,04±0,006 p_1<0,01 p_2<0,01 p_3<0,01 p_4<0,01	-0,074±0,006 p_1<0,01 p_2<0,001 p_4<0,01	-0,02±0,002	-0,04±0,004
Catalase, activity units	6,70±0,32 p_1<0,01 p_2<0,01 p_3<0,01 p_4<0,01	4,70±0,55 p_1<0,01 p_2<0,01 p_4<0,01	8,97±0,24	7,10±0,32

Note: p_1 – as compared to control group with healthy periodontium, p_2 – as compared to control group with periodontal disease, p_3 – as compared to LGM group with periodontal disease, p_4 – as compared to LGM 2 group.

lower in the LGM 2 group with periodontal disease (5,1 units) as compared to the healthy children with periodontal disease (p<0,001), but higher compared to the LGM 2 group without periodontal disease (3,7 units, p<0,01) (Table III). This parameter increased in the LGM 3 group (p<0,01) (Table IV), however, catalase activity was lower in LGM children without periodontal disease (6,7 units) and with it (4,7 units) as compared to parameters of the LGM 2 group (p<0,01) and the control group (p<0,01).

DISCUSSION

The effect of malignant diseases on the periodontal state has been studied in a lot of papers, however, only one research dealt with periodontal changes in patients with Hodgkin`s lymphoma, showing that Russel periodontal index was insignificantly higher in such patients at the remission phase [5]. In the current study, the children with LGM demonstrated a significantly higher frequency of periodontal disease as compared with the healthy children, which increased over the course of

disease. Results close to these in the presented study were obtained by Kaskova et al. [13] in children with acute lymphoblastic leukemia.

Chronic catarrhal gingivitis in healthy children causes an increase of lipid peroxide oxidation parameters and a decrease in the enzymatic activity of antioxidant protective system in oral fluid [14]. However, periodontopathogenic bacteria develop different factors against peroxidation and some anaerobic oral bacteria, including *Streptococcus* species, make use of superoxide dismutases and other enzyms to metabolize oxygen into less harmful derivatives [15], moreover, cariogenic bacteria developed mechanisms of bacterial acid-tolerance [16].

In this study, TBARS level and SOD activity in the healthy children with periodontal disease, represented mostly by gingivitis, was higher than in the counterparts without periodontal disease ($p<0,01$). Results closed to our dates were obtained by Vinnichenko et al. who showed that SOD in saliva increased in patients with mild periodontitis, but decreased at severe stage of its manifestation [17]. Therefore, increased SOD activity could be explained by adaptation mechanism to periodontal disease development. In this study, catalase activity was lower in the children with periodontal disease ($p<0,01$), that agrees with data of Gharbi et al. [18] who showed that patients with periodontitis exhibited a significant decrease in the activities of catalase.

Genetic variation in oxidative stress genes in tumor cells suggests a possible role for oxidative stress in the risk of non-Hodgkin lymphoma [19]. In this study, TBARS level increased over the course of disease, that probably resulted from the decreased immunity of children with LGM and the cytotoxic effect of chemotherapy or radiotherapy. Children had the highest SOD activity and the lowest catalase activity one month after therapy, however, these parameters improved at the remission phase. Glorieu et al. [20] demonstrated that catalase expression is altered in cancer cells, providing their resistance to peroxidation and cytostatics. Skórska et al. [21] showed that higher SOD in serum could be predictor of survival in patients with cancer. So, continued increase of SOD activity with LGM development might be a sign of the remission. However, Tome et al. [22] claimed that an increase of catalase and SOD in lymphoma cells by chronic oxidative stress exposure results in cells with a chemoresistant phenotype. Thus, a role of oxidative stress in pathogenesis of LGM is complicated and has not studied completely yet.

Moreover, presence of periodontal disease in this study altered lipid peroxidation and antioxidant protection in saliva of the children with LGM. These children had significantly higher TBARS level and SOD activity and lower catalase activity compared to their counterparts without periodontal disease ($p<0,01$).

Sum up, both LGM and periodontal disease altered lipid peroxidation and antioxidant protection in saliva of the children. Future enhanced studies may better explicate mechanisms of antioxidant activity changes in patients with periodontal disease related to LGM. To minimize manifestations of periodontal disease related to LGM and its treatment it might be prospective to recommend medicines which regulate antioxidant activity to improve patient`s quality of life.

CONCLUSIONS

6-11 years old children before hematologic therapy showed a higher frequency of periodontal disease (50,0%), as well as 12-15 year olds (80,8%) compared to the control group (17,4% and 42,8% accordingly, $p<0,05$); morbidity increased during next examinations ($p<0,01$).

TBARS levels were higher in LGM children with periodontal disease before and 1 month after hematologic therapy, and at the remission period (9,79, 12,3 and 12,6 umol/l accordingly) as compared to counterparts without periodontal disease (8,01, 10,1 and 11,6 umol/l accordingly, $p<0,01$) and healthy children with periodontal disease (7,9 umol/l, $p<0,01$). SOD activity was higher in LGM 1-3 groups of children with periodontal disease (-0,075, -0,086, -0,074 units accordingly) as compared to counterparts without periodontal disease (-0,048, -0,059, -0,04 units accordingly, $p<0,01$) and healthy children with periodontal disease (-0,04 units, $p<0,01$). Catalase activity was lower in LGM 1-3 groups of children with periodontal disease (6,72, 5,2 and 6,7 units accordingly) as compared to counterparts without periodontal disease (7,3, 3,7 and 4,7 units accordingly, $p<0,01$) and healthy children with periodontal disease (7,1 units, $p<0,01$).

Both LGM and periodontal disease altered lipid peroxidation and antioxidant protection in saliva of the children.

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ORIGINAL ARTICLE

PROTECTIVE EFFECT OF EPROSARTAN IN RENAL ISCHEMIA REPERFUSION INJURY BY REGULATING OXIDATIVE STRESS, INFLAMMATION, AND APOPTOTIC CASCADES IN A BILATERAL RAT MODEL

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ABSTRACT

The aim: To evaluate the potential protective effect of Eprosartan (ARB) in bilateral renal IRI in male rats.

Materials and methods: 20 Sprague-Dawley rats divided into four groups. Sham group had surgery without IRI. Control group was subjected to 30 min ischemia and 2 hours of reperfusion. Vehicle group received 14 ml/kg (IP) injection of solvent mixture containing (10% DMSO, 40% PEG300, 5% Tween-80, and 45% normal saline) 30 minutes before clamping. Eprosartan-treated group with 30 mg/kg Eprosartan intraperitoneally 30 min before occlusion of renal pedicles followed by 30 minutes of ischemia and 2 hours of reperfusion. Serum BUN and Creatinine used to assess renal function. Renal tissue was used to measure the levels of TNF-α, IL-1β, IL-6, F2-isoprostane, and Caspase3 were measured by assessment of renal tissue. Histopathological examinations were conducted to detect parenchymal damage.

Results: Mean serum levels of BUN and Creatinine as well as mean renal tissue levels of TNF-α, IL-1β, IL-6, F2-isoprostane, and Caspase3 were significantly increased in control and vehicle groups together with increase in histological damage score compared to sham group, whereas treatment of rats with Eprosartan resulted in significant reduction in mean serum levels of BUN and Creatinine and mean renal tissue levels of TNF-α, IL-1β, IL-6, F2-isoprostane, and Caspase3 and obvious reduction in tissue injury.

Conclusions: This study demonstrates that Eprosartan pretreatment enhances kidney function by decreasing serum BUN and Creatinine, oxidative stress, cytokines, and apoptotic markers.

KEY WORDS: effect of Eprosartan, oxidative stress, apoptotic cascades, bilateral rat model

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INTRODUCTION

Ischemia/reperfusion injury (IRI) is a term used for describing the functional and structural changes that occur when blood flow is restored after a period of ischemia· Comes as a result of restriction of blood flow to an organ, followed by rejuvenation of blood flow and re-oxygenation. Injuries are inevitable and may occur following infarction, renal artery stenosis, sepsis, partial nephrectomy, and, most frequently, during kidney transplantation [1]. Ischemia-reperfusion injury is characterized by pathophysiological mechanisms with both local and systemic effects. This process occurs basically in two stages: first, during ischemia, cell energy depletion is the primary factor, and second, during reperfusion, the interface events of oxidative and microcirculatory stress, as well as inflammation and apoptosis [2]. Injury mechanisms usually involve the production of reactive oxygen species (ROS) response to hypoxia and reperfusion, and pro-inflammatory cytokines, chemokines, as well as the activation of multiple enzymes and leukocyte activation and neutrophil infiltration which impairs organ function and aggravates tissue damage [3]. Renal injury due to ischemia/reperfusion (I/R) occurs predictably in situations where the kidneys are deprived of blood supply for a period of time, followed by resumption of blood flow and oxygenation of the tissue, as it can be shown in patients who have undergone kidney transplantation, trauma, shock, burn, vascular surgery and the unavoidable outcomes of organ transplantation and cardiovascular surgery involving aortic clamping. IRI is also one of the leading causes of acute kidney injury, which affects approximately 13 million people worldwide [4]. Acute kidney injury (AKI) is a significant

and critical problem for nephrology consultants and a common clinical situation in intensive care settings in a hospital setting patients and is implicated in high mortality rates. AKI is expressed by a rapid decline in kidney function. AKI can cause damage to organs other than the kidneys, including the heart, lungs, liver, intestines, and brain [5-7]. Ischemia-reperfusion (I/R) injury induces a molecular and cellular inflammatory response in the kidney, including the activation of the inflammatory-relevant transcription factor nuclear factor-kappa B (NF-κB), which plays a pivotal role in the pathogenesis of I/R injury, which results in elevation of pro-inflammatory cytokines like interleukin 1-beta (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor (TNF-α) [8-10]. The inflammatory process is initiated by the dysfunction of both endothelial and tubular cells. IL-1, IL-6, and IL-8, TNF-α, TGF-β and MCP-1 are among the proinflammatory/immunomodulatory cytokines released through into renal tissue and circulation. The release of IL-6 enhances the expression of oxidative stress and the extent of kidney damage, malfunction, and inflammation [11-13]. IL-6 signaling occurs following the binding of IL-6 to IL-6 receptor, which activates the signal transducer and activator of transcription 3 (STAT3). The inflammatory cytokines, which include TNF-α and IL-1β, are small proteins that mediate and regulate inflammation. Tissue I/R damage activates cascades, which upregulated cytokines such as TNF-α and IL-1β and plays a crucial role in the initiation of the systemic inflammatory response. Moreover, neutrophil accumulation and the level of cytokines containing TNF-α and IL-1β were higher in the reperfused kidney while renal TNF-α expression mediates neutrophil infiltration and injury after renal I/R. The transcription component Nuclear Factor-kappa B (NF-κB) is a crucial component in the regulation of innate immunity in ischemic tissues, playing a significant role in helping to promote the expression of diverse genes, which include proinflammatory genes (such as chemokines and cytokines) and genes related to cell adhesion and growth control [14]. Considered a novel target for the treatment of inflammatory diseases. TNF-α triggers activation of the NF-κB, The promoter region of the TNF-α gene has several DNA binding sites for the transcription protein nuclear factor kappa B (NF-κB), suggesting that NF-κB is essential for TNF-α production. Oxidative stress is a state that occurs when Inadequate balance exists between the formation of reactive oxygen species (ROS) also called free radicals, which has one or more unpaired electrons and is therefore chemically highly reactive; occurs by increasing (superoxide anions ($O2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide (H2O2)), and decreasing in antioxidant defense system (SOD, GPx, and Catalase) [15]. Recent research shows that abnormal apoptosis and endoplasmic reticulum stress (ERS) of renal tubular epithelial cells may influence the occurrence and progression of acute kidney injury (AKI). Activation of the Janus kinase/signal transducer and activator of transcription (JAK2/STAT3) pathway mediated the production of numerous pro-inflammatory cytokines that contributed to the progression of renal IRI. Several efforts were made to demonstrate that angiotensin II, upon activation of the AT_1 receptor, has a strong relation with the Janus kinase2/signal transducers and activators of transcription3 (JAK2/STAT3) signaling pathway and induces the phosphorylation of JAK2 and subsequent phosphorylation of STAT3 [16]. Renal IRI induces an inflammatory cascade that contributes to further renal damage; therefore, inhibition of inflammatory responses is a therapeutic strategy for protecting renal tissue. F2-isoprostanes are an end-product biomarker that can be used to assess systemic as well as renal oxidative stress. It considered by some scientists to be the best accurate available biomarker of lipid peroxidation oxidative stress in chronic kidney disease (CKD). Eprosartan (EPR) is an Angiotensin-receptor blocker that selectively antagonizes the angiotensin II type 1 (AT1R) receptor and counteracts most of the deleterious actions of angiotensin II. Eprosartan is an ARB with a special chemical structure that may be relevant to its mechanism of action [10, 17]. One of the most promising angiotensin II receptor antagonists is EPR [16]. EPR a potent AT1R receptor antagonist differs from other angiotensin receptor blockers (ARBs) by chemical distinction and has a unique dual mechanism of action. It not only blocks AG II but provides combined inhibition of both the RAS and sympathetic nervous system by inhibiting sympathoadrenal activation by angiotensin II at the presynaptic level [18-19]. EPR exerts beneficial effects on the vasculature by inhibiting mechanisms of inflammation and oxidation.

MATERIALS AND METHODS

ANIMAL MAINTENANCE AND PREPARATION

Animals were fed in a standard diet of food with water this experimental study was conducted in the research unit of the College of Medicine at Kufa University and the laboratory of the Pharmacology and Therapeutics branch of the College of Medicine at Kufa University. They were bought from the Ministry of Health's Center for Control and Pharmaceutical Research in Baghdad and housed in the animal facility at the university's Animal Resources Centre in the College of Sciences, Kufa University, with a temperature regulates at 24±2°C and

humidity amongst the 60-65 percent in conformity with the pattern 24-hour dark/light cycle. After receiving permission from the Institutional Animal Care and Use Committee (IACUC) at the University of Kufa, all the rats were enrolled in the study.

EXPERIMENTAL DESIGN

Sprague Dawley rats ranging in age from 20 to 24 weeks and weight from 200 to 350 gm were used. Animals were assigned to four groups (5 animals in each group). The sham group underwent surgical procedures without IRI induction. The I/R control group was subjected to ischemia by clamping the renal pedicles for 30 minutes, followed by blood restoration for 2 hours [20-21]. The vehicle treated group received 14 ml/kg of an intraperitoneal (IP) injection of a solvent mixture containing 10% DMSO, 40% PEG300, 5% Tween-80, and 45% saline 30 minutes prior to clamping. Eprosartan-treated group: rats pretreated with 30 mg/kg Eprosartan intraperitoneally half an hour prior to occlusion of renal pedicles. The animals were then subjected to 30 minutes of ischemia by clamping of renal pedicles followed by 2 hours of reperfusion.

INDUCTION OF RENAL IRI

All rats were weighed and anesthetized with an intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine [22], under sedation (5-10 minutes), in order to keep the rats stable during surgery, their limbs and tails were taped down after they were laid on their backs. The chest and abdomen were shaved, and the skin was disinfected with a 10% iodine spray. By pinching the tail and hind feet, it was determined whether the rats were sufficiently anesthetized. The intestine was retracted to expose the abdominal cavity and both renal pedicles and a midline laparotomy incision is made. Incorporating a Model of Bilateral Ischemia, The renal pedicles were then isolated, and non-traumatic micro vascular clamps were placed around the renal pedicles to clamp the renal artery and vein [23]. Occlusion was confirmed by observing patchy blanching of the entire kidney surface, which, after several minutes, changes the color of a kidney from red to dark purple. The total clamping time with non-traumatic vascular clamps was 30 minutes. During this procedure, 1 ml of normal saline was injected into the abdomen, and then the abdomen was covered with warm and moist gauze to ensure that the animals remained adequately hydrated. Throughout the experiments, the body temperature of each rat was monitored and maintained at 37°C. After thirty minutes, the clamps were removed from the pedicles, restoring renal blood flow, and initiating the reperfusion phase. The kidney was repositioned, and the abdominal cavity incision was closed in two layers with three interrupted sutures. After that, the rat was euthanized by deep anesthesia, lastly, blood and tissue samples were collected for examination. It is important to note that Kidney ischemia lasting longer than 20 minutes is associated with a renal injury in rat model.

EPROSARTAN PREPARATION

The pure powder of Eprosartan mesylate (Pub Chem CID: 5281037) was purchased from Med Chem Express, USA Company. Molecular Formula: $C_{24}H_{28}N_2O_7S_2$ and Chemical Name: [(E)-alpha-[[2-butyl-1-[4 carboxyphenyl methyl]-1H-imidazol-5-yl] methylene]-2thiophenepropanoic acid CAS No.: 144143-96-4, Cat. No.: HY-15834A, Purity: physical description: a crystalline solid, Solubility In the present study, to prepare the drug, Eprosartan mesylate powder was dissolved in these solvents each one by one: 10% DMSO, 40% PEG300, 5% Tween-80, 45% saline, respectively with Solubility of ≥2.08 mg/mL, Clear solution, according to manufacturer datasheet instructions, Med Chem Express, USA Company. The dose of the drug that was used is 30 mg/kg of rat weight intraperitoneally.

ASSESSMENT OF THE RENAL FUNCTION BY COLORIMETRIC METHOD

After the procedure is complete, each rat's heart was punctured, and about (3.5-5 ml) of blood was withdrawn while the animal was under anesthesia [24]. Each sample of blood was stored in a gel tube at 37°C without anticoagulant for 30 minutes. Ahead of time, we labeled and stacked each gel tube in a rack, The samples were gathered, and centrifuged at 3000 rpm for 10 minutes at 4°C, and the sera were stored at 80°C before being used to measure urea and creatinine concentrations. Serum samples were analyzed for serum BUN and serum Creatinine (Scr.) using colorimetric methods, and commercial kits according to the manufacturer's instructions to determine renal functional parameters.

TNF-α, IL-1β, IL-6, Caspases 3 and F2-isoprostane measurement in kidney using ELISA.

Immediately following the removal of the rat's heart, the left kidney was removed, and the organ was washed in ice-cold isotonic normal saline 0.9% to remove any blood clots before being dissected in half. One section was homogenized using a high-intensity ultrasonic liquid processor in a 1:10 (w/v) dilution of phosphate-buffered saline (PBS) containing 1% Triton X-100 and a protease inhibitor cocktail. The homogenate samples

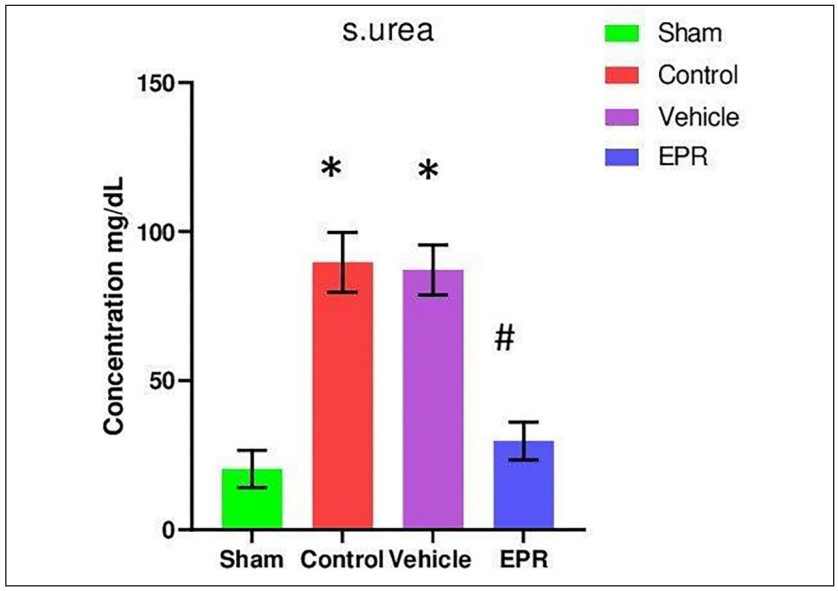

Fig. 1. Mean serum BUN level amongst the groups.
The data are expressed as mean±SEM, N=5. *: p ≤0.01 compared to the sham group; #: p ≤0.01 compared to I/R control and vehicle treated groups; EPR: Eprosartan

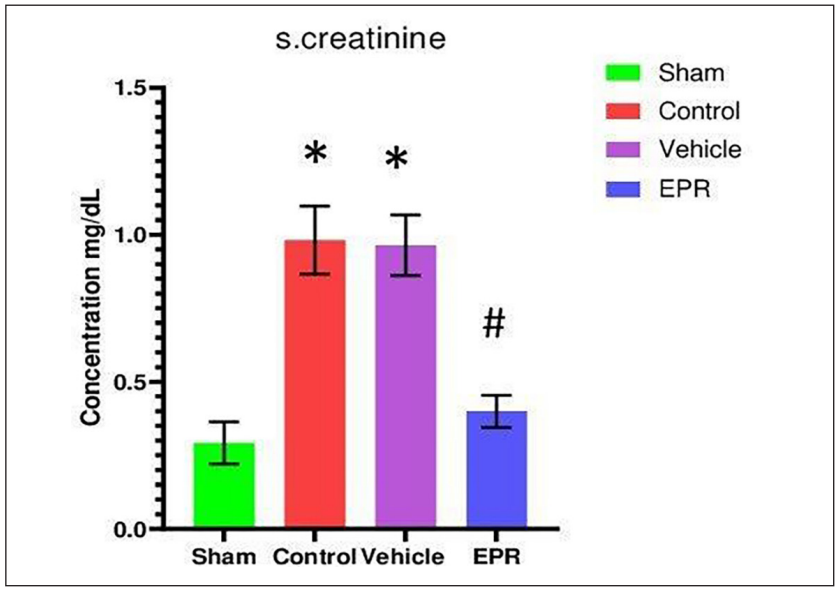

Fig. 2. Mean serum creatinine level amongst the groups.
The data are expressed as mean±SEM, N=5. *: p ≤ 0.01 compared to the sham group; #: p ≤ 0.01 compared to I/R control and vehicle treated groups; EPR: Eprosartan.

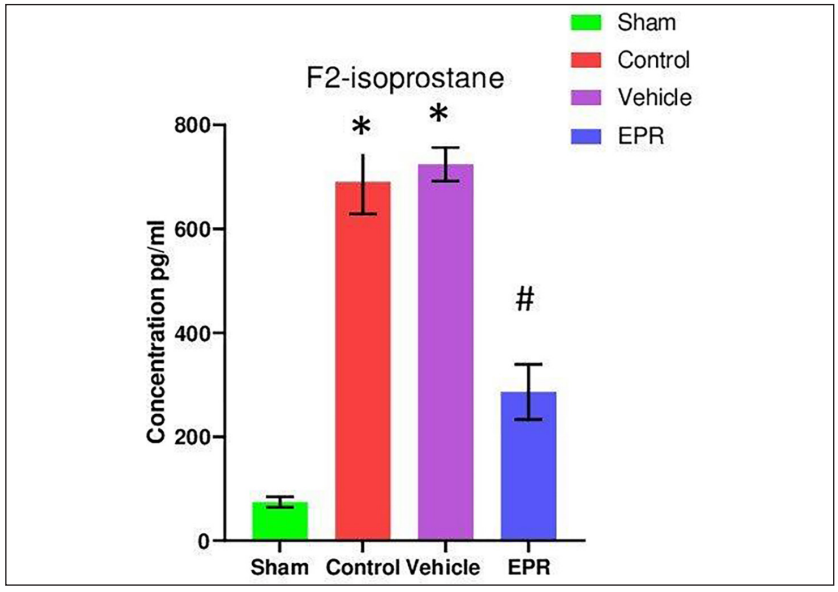

Fig. 3. Mean F2-isoprostane renal tissue level amongst the groups.
The data are expressed as mean±SEM, N=5. *: p ≤0.01 compared to the sham group; #: p ≤0.01 compared to I/R control and vehicle treated groups; EPR: Eprosartan.

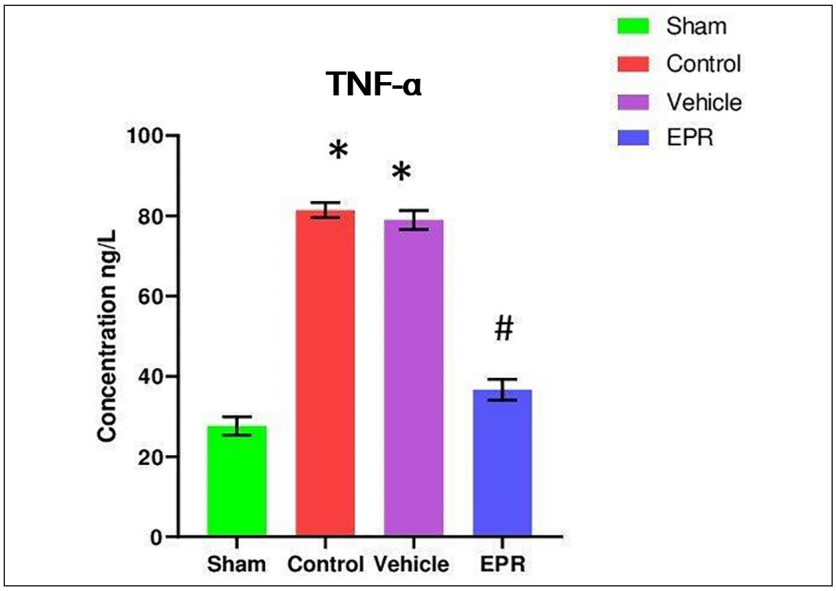

Fig. 4. Mean TNF-α renal tissue level amongst the groups.
The data are expressed as mean±SEM, N=5. *: p ≤ 0.01 compared to the sham group; #: p ≤ 0.01 compared to I/R control and vehicle treated groups; EPR: Eprosartan

were centrifuged at 4°C for 15 minutes at 14000 rpm [24]. In accordance with the manufacturer's instructions for the ELISA kits used (Bioassay Technology Laboratory), the supernatant was collected and analyzed for TNF-α, IL-1β, IL-6, Caspases 3, and F2-isoprostane levels by ELISA technique. (Bioassay Technology Laboratory, China) [25].

TISSUE SAMPLING FOR HISTOPATHOLOGY ANALYSIS AND DAMAGE SCORES

Analogously, the other portion of the left kidney tissue sample was fixed in 10% formalin, dehydrated in alcohol series, cleared in xylene, and embedded in a paraffin block. Tissue slides were cut into horizontal 5-µm thick sections using a microtome, stained with Hematoxylin and Eosin for morphological assessment and histological scoring [24], and then sent to a histopathologist for a histopathological evaluation. After fixation, an observer who was blinded to the experimental treatment groups evaluated scores. Tissue sections were examined by light microscopy and graded for degeneration/necrosis. The scoring system of tissue damage assessment using quantitative measurements. Renal tubular damage is characterized by tubular epithelial swelling, increased cytoplasmic eosinophilia, necrotic tubules, loss of brush border, vacuolar degeneration, and Eosinophilic cast formation. The degree of kidney injury was estimated at X100 and X400 magnifications [25]. The score of histological changes was identified as a percentage of renal tubular damage in the section of kidney tissue as follows:

- score 0, represents normal,
- score 1, represents ≤25% of tubular damages,
- score 2, represents 25-50% of tubular damages,
- score 3, represents 50-75% of tubular damages,
- score 4, represents >75% of tubular damages.

STATISTICAL ANALYSIS

Statistical analysis of the experimental results was conducted according to GraphPad Prism version 7.0, where Tukey multiple comparisons One-way (ANOVA) was performed to investigate the significance of differences between groups. The values were expressed as mean ± standard errors of the mean (SEM) and a P value ≤0.01 was considered statistically significant.

RESULTS

IMPLICATIONS OF I/R AND EPROSARTAN ON PARAMETERS OF KIDNEY FUNCTION (SERUM BUN AND SERUM CREATININE)

This investigation divulged that the serum levels of BUN and Scr. in the sham group were found to be significantly (p ≤0.01) reduced compared to the levels in I/R control and vehicle groups. Comparison of I/R control and vehicle groups showed no statistically significant difference (p >0.01). Serum BUN and Scr. levels were significantly (p ≤0.01) reduced in the Eprosartan treated group compared to both I/R control and vehicle groups. Differences in the serum BUN and Scr. levels are documented in Figures 1 and 2.

Implications of I/R and Eprosartan on the oxidative stress marker in the kidney (F2-isoprostane)

This investigation divulged that the renal tissue level of F2-isoprostane in the sham group was found to be significantly (p ≤0.01) reduced compared to the levels in I/R control and vehicle groups. Comparison of I/R

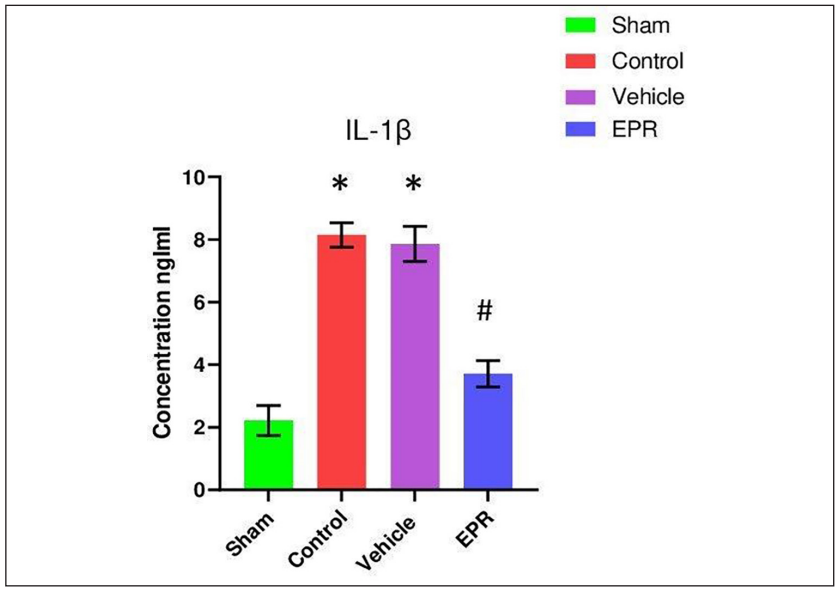

Fig. 5. Mean IL-1β renal tissue level amongst the groups.
The data are expressed as mean±SEM, N=5. *: p ≤0.01 compared to the sham group; #: p ≤ 0.01 compared to I/R control and vehicle treated groups; EPR: Eprosartan.

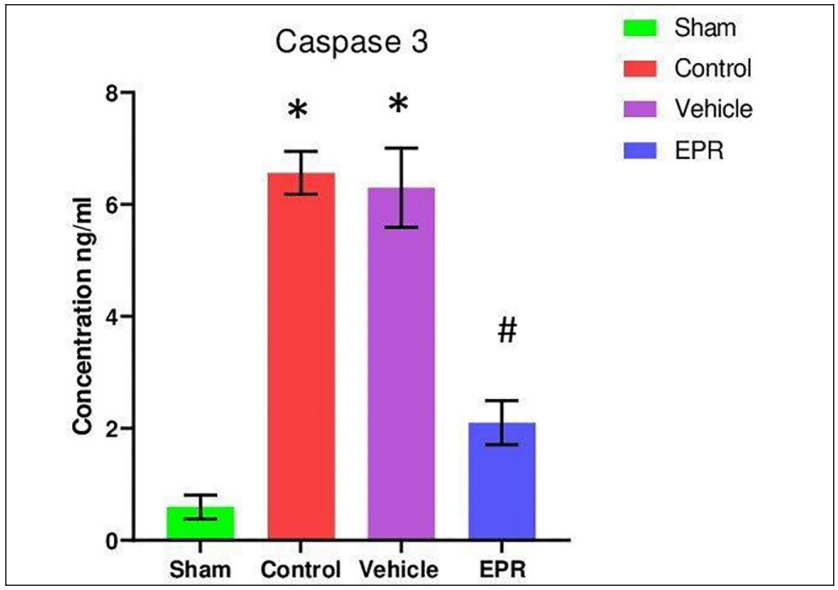

Fig. 6. Mean Caspases 3 renal tissue level amongst the groups. The data are expressed as mean±SEM, N=5. *: p ≤0.01 compared to the sham group; #: p ≤0.01 compared to I/R control and vehicle treated groups; EPR: Eprosartan.

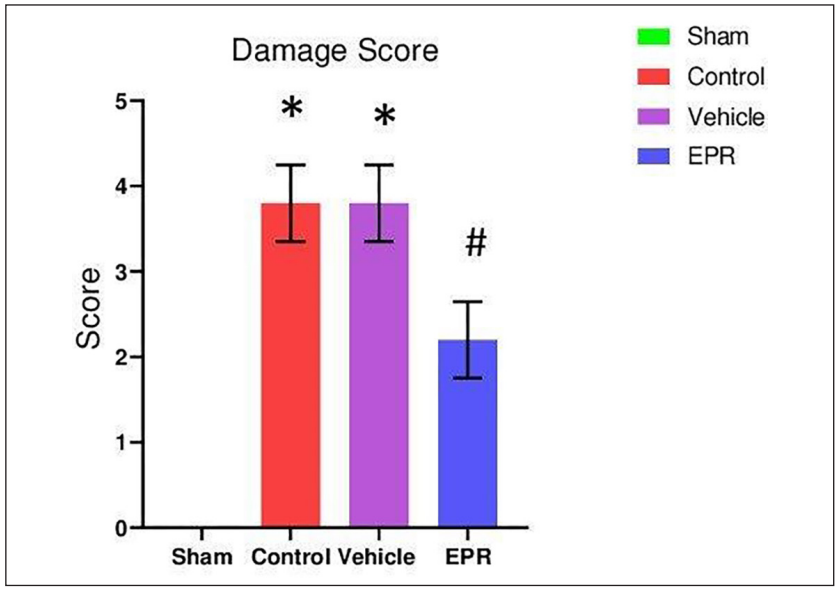

Fig. 7. Mean histopathology scores amongst the groups.
The data are expressed as mean±SEM, N=5. *: p ≤ 0.01 compared to the sham group; #: p ≤ 0.01 compared to I/R control and vehicle treated groups; EPR: Eprosartan

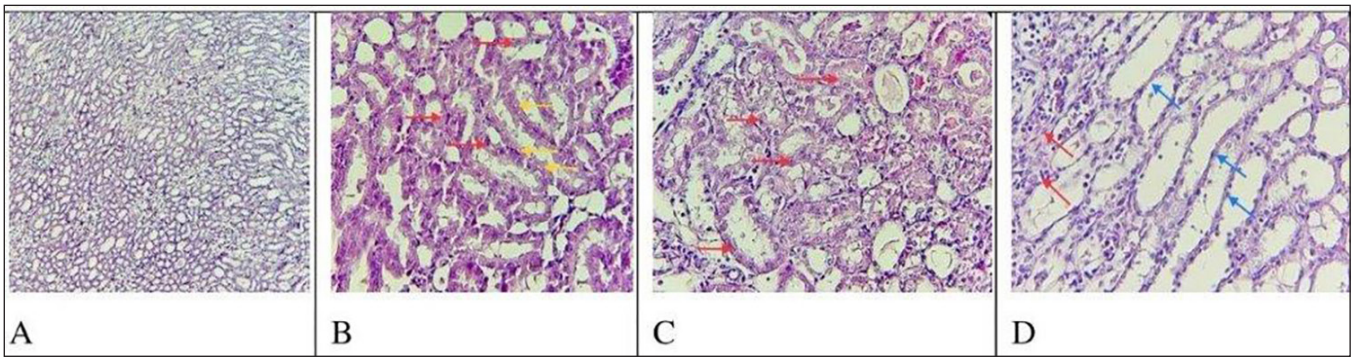

Fig. 8. Sections of renal tissue stained with hematoxylin and eosin, are depicted in the images above. A) Sham group showed shows normal histology of renal tubules; B) Control group shows cellular swelling, and increased cytoplasmic eosinophilia) red arrows), cytoplasmic vacuoles (yellow arrows); C) Vehicle treated group shows a cellular injury including cellular swelling, increased cytoplasmic eosinophilia (red arrows), Eosinophilic cast (blue arrows); D) Eprosartan treated group shows damaged renal tubules (red arrows), normal renal tubules (blue arrows).

control and vehicle groups showed no statistically significant difference ($p > 0.01$). Renal tissue level of F2-isoprostane was significantly ($p \leq 0.01$) reduced in the Eprosartan treated group compared to both I/R control and vehicle groups. Differences in renal tissue level of F2-isoprostane levels are documented in Figure 3.

Implications of I/R and Eprosartan on the markers of inflammation in the kidney (TNF-α, IL-1β).

This investigation divulged that the renal tissue level of TNF-α and IL-1β in the sham group were found to be significantly ($p \leq 0.01$) reduced compared to the levels in I/R control and vehicle groups. Comparison of I/R control and vehicle groups showed no statistically significant difference ($p > 0.01$). Renal tissue level of TNF-α and IL-1β was significantly ($p \leq 0.01$) reduced in The Eprosartan treated group compared to both I/R control and vehicle groups. Differences in renal tissue level of TNF-α and IL-1β levels are documented in Figures 4 and 5.

Implications of I/R and Eprosartan on the marker of apoptosis in the kidney (caspases 3).

This investigation divulged that the renal tissue level of caspases 3 in the sham group was found to be significantly ($p \leq 0.01$) reduced compared to the levels in I/R control and vehicle groups. Comparison of I/R control and vehicle groups showed no statistically significant difference ($p > 0.01$). Renal tissue level of caspases 3 was significantly ($p \leq 0.01$) reduced in the Eprosartan treated group compared to both I/R control and vehicle groups. Differences in renal tissue level of caspases 3 levels are documented in Figure 6.

HISTOPATHOLOGICAL EXAMINATION

The damage score and architectural changes of kidneys among the groups were shown in Figures 7 and 8. The renal architecture was normal in the sham group (Fig. 8A). Conversely, the histopathological investigation in I/R control and vehicle treated groups abnormality in renal structure and severe renal damage including cellular swelling, increased cytoplasmic eosinophilia, and loss of brush borders. Moreover, a cast formation and congestion of the lumen are observed. (Fig. 8B and 8C). Eprosartan pretreated group preserved kidney architecture normally. The treatment effect is characterized by a slight swelling of the renal tubules with mild interstitial congestion (Fig. 8D).

DISCUSSION

This study revealed that significant increase in serum BUN and Scr. levels in I/R control and vehicle treated groups in comparison to the sham group. Low renal blood flow and, consequently, a decline in glomerular filtration rate due to renal tissue injury contribute most to the rise of these two renal functional parameters [26]. The current investigation divulged that the pre-treatment with AT_1 receptor blocker Eprosartan prior to ischemia induction is significantly ($p \leq 0.01$) lowering the serum levels of BUN and Scr. compared to levels in both I/R control and vehicle groups. This result indicates that Eprosartan has a protective effect on parameters of renal function (BUN and Scr) after renal IRI was induced in a rat model. This finding agrees with other studies. Eprosartan, in an experimental study on rats, was found to reduce serum BUN and Scr. levels and protect renal function from the toxicity of streptozotocin, which can evoke diabetic nephropathy and produce a nephrotoxic effect and uplift serum BUN and Scr. when given alone. Research shows that one of the reasons an ARB is effective in treating kidney disease is due to lowering the blood pressure by relaxing blood vessels systemically, additionally, ARB-induced renal vasodilation improves renal ischemia and hypoxia by increasing renal blood

flow [27]. The histopathological findings of the present study showed a significant decrease in the degree of tissue injury in the Eprosartan treated group in comparison to I/R control and vehicle treated groups. This finding was reported by a previous study which showed that, when given prior to ischemia, Eprosartan protects the kidneys by reducing the severity of renal injury through its anti-inflammatory and antioxidant effects and this lowering in the degree of injury may be related to Eprosartan ability to reduce the degree of injury as a result, fewer inflammatory cells are recruited to areas of tissue damage. Previous studies have noted that inflammatory cytokines such as TNF-α and IL-1β were increased in a rat model of renal ischemic and causes deleterious effects. Moreover, the increase in these inflammatory cytokines is the result of hypoxia followed by a reduction in renal blood flow. Increased levels of inflammatory cytokines cause an influx of the infiltration of inflammatory cells into damaged tissue [28]. The data gathered in this experiment prove that pre-treatment with the AT_1 receptor blocker Eprosartan prior to ischemia induction reduces the level of inflammatory mediators (TNF-α and IL-1β) in renal ischemic tissues, as compared to the levels of these inflammatory cytokines in the IR control and vehicle groups. This signifies that Eprosartan inhibits inflammation in kidney tissues that have been subjected to ischemia and reperfusion. Our exploration is consistent with other experimental studies. In a study, Eprosartan was found to have Nephroprotective effects against I/R-induced kidney damage in rats by decreasing inflammatory pathways. A possible explanation for this might be demonstrated in these studies. A study revealed that Eprosartan has a Neuroprotective mechanism in focal cerebral ischemia via blocking AT_1 receptors, considerably reducing the P-JAK2 and P-STAT3 levels, one of the most significant inflammatory pathways in the rat hippocampus. P-JAK2 and P-STAT3 levels rise after cerebral ischemia, resulting in multiple neuroinflammatory and apoptotic events. A study revealed that inhibiting the activation of the JAK2/STAT3 pathway protected rats from acute renal injury [29]. Locally produced angiotensin II in the kidney, in addition to its hypertensinogenic effect, activates numerous intracellular signaling pathways and induces inflammation, renal cell growth, mitogenesis, apoptosis, migration, and differentiation. These effects of angiotensin II are also mediated through AT_1R receptor activation and play an important role in the pathogenesis of renal tissue injury. Furthermore, this study reported a significant increase in F2-isoprostane in renal tissues after IRI in both I/R control and vehicle groups compared to the sham group. This means that there is an increase in oxidative stress and ROS formation in injured renal tissues of I/R control and vehicle groups compared to the intact tissues of the sham group. Our result is consistent with previous research. Several studies showed that the increase in oxidative stress and the elevation in ROS formation are considered to be the primary pathological mechanisms in renal IRI. During the process of blood reperfusion into ischemic tissues, a substantial amount of ROS is produced, which exacerbates ischemic injury and renal dysfunction. Due to their rapid metabolic rate, it was reported that renal tubular cells are susceptible to oxidative stress. A study showed that the massive production of ROS leads to inflammation status capable of infiltration of neutrophils, promoting DNA damage, increased secretion of protease, and the production of a large number of oxidative intermediates.as well as the upregulation of growth factors, cytokines and genes involved in cell survival, underlining their impact on several signaling pathways and mechanisms. This study demonstrated that the AT_1 receptor blocker Eprosartan significantly decreased the F2-isoprostane level and oxidative stress and free radical formation in ischemic renal tissues when compared to I/R control and vehicle groups. This indicates that Eprosartan has an antioxidant effect on injured and inflamed kidney tissues undergoing I/R. One study divulged that Pre-treatment with Eprosartan decreased oxidative and apoptotic mediators in the hippocampus of rats. Eprosartan exerts beneficial effects on the vasculature by inhibiting mechanisms of inflammation and oxidation. This result is in agreement with a study that showed that Eprosartan enhances the antioxidant system by increasing GSH levels, GPx, and SOD activities. This experimental study reported that there is a significant elevation in caspases 3 levels in renal tissue of both I/R control and vehicle groups ($p \leq 0.01$) compared to the sham group after IRI. Caspase-3 (cysteine protease) is an important enzyme involved in inflammation and apoptosis, key features of renal IR injury. Multiple inflammatory mediators, neutrophil infiltration, caspases activation, and the presence of apoptosis may be responsible for the progression of I/R injury-related inflammation. Furthermore, caspases has the potential to influence the development of inflammation and the associated changes in renal function and morphologic structure according to [30], it was found that the renal tissue level of caspases 3 is significantly ($P \leq 0.01$) decreased in AT1 receptor blocker Eprosartan treated group in comparison with I/R control and vehicle groups. This result is in agreement with a study that showed that Eprosartan markedly decrease levels of caspases 3 in comparison with the untreated group in the renal I/R rat model and this may be related to the anti-apoptotic effect of Eprosartan. A

study revealed that blocking the AT1 receptor in the heart inhibits apoptosis by suppressing the action of angiotensin II, and this may be linked to the protective effects of Eprosartan against caspases 3 that blocks the AT1 receptor in the kidney.

CONCLUSIONS

This study demonstrates that Eprosartan pretreatment enhances kidney function by decreasing serum BUN and Creatinine, oxidative stress, cytokines, and apoptotic markers.

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ORIGINAL ARTICLE

PECULIARITIES OF FORMATION OF CARBOHYDRATE METABOLISM DISORDERS WITH COMORBID CHRONIC PANCREATITIS AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

The aim: To determine glycemic condition, regulation of carbohydrate metabolism, degree of insulin resistance in patients with chronic pancreatitis with its isolated course and with comorbid COPD and diabetes mellitus.

Materials and methods: 110 patients with chronic pancreatitis were examined. The first group of patients included 38 individuals with an isolated course of chronic pancreatitis (1 group), 2nd group included 35 patients with chronic pancreatitis and COPD, 3rd group included 37 patients with chronic pancreatitis and COPD and type 3c diabetes mellitus. The control group (CCOPD) included 32 individuals with isolated COPD, the control group (CDM) includes 34 individuals with isolated type 2 diabetes mellitus. All the patients were examined for functional state of the pancreas and carbohydrate metabolism was assessed.

Results: Patients suffering from chronic pancreatitis with COPD and diabetes mellitus developed 3.2 times increased glucose concentration on an empty stomach. Blood glucagon content in all patients was lower in comparison with that of practically healthy individuals which is indicative of an insufficient glucagon secretion by α-cells of the pancreas. Pancreatic polypeptide content in the blood was lower in patients with chronic pancreatitis and COPD and T3c diabetes mellitus in comparison with the reference value.

Conclusions: A comorbid course of chronic pancreatitis with exacerbated COPD is associated with more intensive disturbances in carbohydrate metabolism regulation and glycaemia parameters in comparison with an isolated course of chronic pancreatitis. In case comorbidity includes a chronic pancreatitis, chronic obstructive pulmonary disease and diabetes mellitus, the most unfavorable glycemic profile is found which is indicative of carbohydrate metabolism decompensation.

KEY WORDS: chronic pancreatitis, diabetes mellitus, insulin, chronic obstructive pulmonary disease, glucagon, pancreatic polypeptide

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INTRODUCTION

Chronic pancreatitis (CP) of various etiology and chronic obstructive pulmonary disease (COPD) are a frequent association of somatic pathology in the internal medicine clinic [1-4]. This combination is rather specific since recently the sickness rate of both diseases has increased. Moreover, there are many mechanisms mutually compromising these pathologies, severity and resistance of patients to the programmatic therapy developed for an isolate course of the diseases are constantly increasing [1, 2]. Each nosology out of this pathological tandem can result in glucose metabolism disorders, since both organs – the pancreas and lungs – take an active part in a direct supply of carbohydrate metabolism and its regulation [3, 5]. Thus, chronic pancreatitis is inflammation of the pancreas associated with the development of pancreatic swelling, its dystrophy, atrophy, fibrosis, strictures of the ducts, calcification of the organ, exocrine and endocrine functional disorders of the pancreas [6]. At initial stages during chronic pancreatitis exacerbations, in addition to enzymatic imbalance of the pancreas hyperinsulinemia with clinical signs of hypoglycemic conditions is observed [7, 8]. In case of chronic pancreatitis developing for many years occurrence of secondary diabetes mellitus (DM) is possible due to a considerable decrease of the area or amount of functioning β-cells in the islet of Langerhans and absolute insulin insufficiency [9]. The case in question is type 3c diabetes mellitus (T3cDM) occurring due to impairment in pancreatic endocrine function as a special nosologic and morphofunctional phenomenon which is not still clearly recognized in the Ukrainian recommendations and classifications [10]. In recent years gastroenterologists have paid special

attention to the issues of this DM variant indicating a close relation of its onset with diseases of the pancreas: several episodes of severe acute pancreatitis, condition after distal pancreatectomy due to pancreonecrosis, alcoholic chronic pancreatitis with an increasing risk of DM development more than three times after beginning of pancreas calcification, hemochromatosis etc. [6, 9-20].

Epidemiological data concerning this type of DM with chronic pancreatitis are unknown now, though there are certain reports indicating its occurrence within 40-60% from the general morbidity associated with chronic pancreatitis [21, 22]. T3cDM is characterized not only by the development of β-cellular insufficiency but decreased glucagon secretion, and association of DM with pancreatic cancer [12, 17].

In 2006 the American Diabetes Association (ADA) expanded DM classification in the chapter "Exocrine diseases of the pancreas" specifying that any process provoking diffuse damage of the pancreas can cause diabetes. The following diseases were considered among the causes: chronic pancreatitis, injury of the pancreas, infection of the pancreas, pancreatectomy, pancreatic adenocarcinoma in the duct, cystic fibrosis and hemochromatosis [9-11]. In the current clinical practice the most frequent causes of T3cDM are chronic pancreatitis (76-79%), pancreatic cancer (8-9%), emochromatosis (7-8%), cystic fibrosis (4%), pancreatectomy (2-3%) [14-16]. In 2011 ADA and the WHO classified DM occurring due to pancreatic diseases as T3cDM [18].

A working group was organized within the frame of the annual Congress studying the issues of the pancreas Pancreas Fest, which in 2012 elaborated a consensus to use the term "T3cDM" [18]. The consensus declared a position that carbohydrate metabolic disorders are common complications of CP with accurate recommendations concerning diagnostics and verification of the diagnosis T3cDM [18]. The Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreatic Cancer (CPDPC) in 2016 recognized the term "T3cDM" [19]. Carbohydrate metabolic disorders with this type of DM vary from hypoglycemia to hyperglycemia on an empty stomach – insulin dependent DM [6, 9-19]. Its clinical manifestation can imitate both type 2 and type 1 DM [18]. The CPDPC consensus emphasizes that even at the initial stages of CP development inflammatory processes and an increase concentration of cytokines in the pancreas parenchyma can lead to dysfunction of β-cells of a various degree [19]. Then insulin secretion decreases progressively with DM secondary to CP. It correlates with a reduced mass of the acinar epithelium and β-cells. Thus, T3c DM is characterized by a various degree of glycemic disorders and metabolism on the whole [14]. Unless special investigations are not completed the CPDPC consensus estimates an average occurrence of T3cDM in the population to be 4-5% (from 1 to 9%) [19, 22, 23].

Peripheral sensitivity to insulin is an urgent issue [4-5, 7]. Certain authors do not consider it to be disturbed [14, 18], others indicate its decrease in pathogenesis of DM with CP to occur more often among obese individuals and in case of hyperlipidemia [17]. The authors of the CPDPC consensus consider pancreatic polypeptide (PP) to be a key factor in pathogenesis of a stable glucose production by the liver and isolated liver resistance to insulin with T3cDM. Pancreatic polypeptide is mostly produced by F-cells of the pancreas – it is polypeptide of 36 amino acid residues [9]. Its secretion is stimulated by parasympathetic cholinergic effects, digestive hormones – ghrelin (a "hunger hormone"), secretin, in case of intake of protein-containing food, during fasting, physical exertion, acute hypoglycemia [6, 9-16]. An increased concentration of pancreatic polypeptide enhances oxygen intake and stimulates sympathetic activity which is indicative of its participation in the energy balance regulation. Its secretion decreases under somatostatin effect and an increasing concentration of glucose in the blood [17-19]. Pancreatic polypeptide inhibits secretion of the pancreatic juice. It is its response to hypoglycemia induced by insulin or mixed-meal tolerance test (MMTT) that is determined as an important differential-diagnostic criterion of T3cDM [12]. Non-responsiveness of pancreatic polypeptide is able to differentiate T3cDM from early type 1 DM, and type 2 DM characterized by an increased content of pancreatic polypeptide in the blood [21].

Therefore, since chronic pancreatitis constitutes about 90% of causes promoting T3cDM, the diagnostics of chronic pancreatitis appears to be an important aspect of early diagnostics of T3cDM. The following diagnostic criteria of chronic pancreatitis with T3cDm complications are recommended [6, 8, 19, 21]: the major (compulsory) and additional. The following criteria are considered to be the major ones: a) exocrine insufficiency of the pancreas available (monoclonal test for fecal elastase-1 or direct functional tests); b) pathological structural changes of the pancreas (its visualization by means of USD, MRI, CT); c) lack of autoimmune markers of type 1 DM. Detection of additional (minor) criteria requires a special study of hormonal markers: 1) lack of pancreatic polypeptide (PP) secretion; 2) disturbances of incretin secretion (glucagon-like peptide-1 (GLP-1)); 3) lack of resistance to insulin (by HOMA-IR); 4) functional disorders of β-cells (HOMA-B, C-peptide/glucose); 5) a reduced content of liposoluble vitamins (A, D, E, K) in the blood [21].

The lungs participate in metabolic processes as well including lipid metabolism. They take exogenous lipids from lipoproteins in the systemic circulation, utilize them for plastic needs, surfactant synthesis, accumulate them in lipocytes and macrophages, and store them for future use to supply energy for all the processes of metabolism and gas exchange [1, 4]. A number of literature sources describe lung participation in glucose metabolism. Thus, such diseases as pneumonia, COPD, bronchial asthma in its exacerbation stage are associated with reduced tolerance to glucose in proportion to increasing hypoxia degree, and development of insulin resistance syndrome [1-2, 4]. At the same time, peculiarities of carbohydrate metabolism compensation in patients with chronic pancreatitis and comorbid COPD and DM are not described in the literature available, they are not studied. Therefore, it has become the aim of our research.

Thus, the working hypothesis of our study was to prove or disprove the likely effect of comorbid COPD on the course of CP and DM, to establish the predominant type of DM in patients with CP with this comorbidity.

THE AIM

The aim of this work was determination of the state of the glycemic profile, regulation of carbohydrate metabolism, the presence and degree of insulin resistance in patients with isolated chronic pancreatitis and the probable interdependence of these parameters on the presence of comorbid COPD and the type of diabetes mellitus.

MATERIALS AND METHODS

A prospective, cross-sectional study was conducted with the analysis of medical records of inpatients in 110 patients with chronic pancreatitis of a mixed etiology in the exacerbation stage of moderate severity were examined. The first group of patients included 38 individuals with an isolated course of chronic pancreatitis (1 group), 2nd group included 35 patients with chronic pancreatitis and COPD GOLD2-3 group D, 3rd group included 37 patients with chronic pancreatitis and COPD GOLD2-3 group D and T3cDM of a moderate severity. The control group (CCOPD) included 32 individuals with isolated COPD GOLD2-3 group D, the control group (CDM) includes 34 individuals with isolated type 2 DM of a moderate severity, subcompensated. The average age of patients was 51.3 ± 3.14. The group of comparison included 30 practically healthy individuals (PHI).

The diagnosis of chronic pancreatitis was made according to the unified clinical protocol approved by the Order of the Ministry of Heath of Ukraine № 638 dated 10.09.2014 «On Approval and Introduction of Medical-Technological Documents Concerning Standardized Medical Aid for Chronic Pancreatitis» on the basis of classical clinical, ultrasonographic, and biochemical methods. COPD was diagnosed and treated according to the recommendations of clinical regularities (the Order of the Ministry of Heath of Ukraine №555 dated 27.06.2013 considering GOLD recommendations, 2019). Distribution of COPD patients into A, B, C, D groups was assessed according to the Modified dyspnea scale of the Medical Research Council and the sum total calculated by COPD assessment test.

On admission to the hospital all the patients were examined for functional state of the pancreas according to the approved list of activity of the pancreatic enzymes in duodenal content, systemic blood circulation and fecal elastase-1 content, proteinogram test, proteolytic intensity of high and low-molecular proteins.

Carbohydrate metabolism was assessed by glucose level on an empty stomach and 2 hours after taking meals by means of glucose-oxidase method, insulin level in the blood on an empty stomach, glucagon on an empty stomach (DRG System), pancreatic polypeptide (ELISA Kitfor Pancreatic Polypeptide (PP)) by means of immune-enzyme analysis (IEA), glycated hemoglobin content in the blood (HbA1c) by means of a standard set of reagents "SimkoLtd" (Lviv) according to V.A. Koroliov's method. Degree of insulin resistance was determined by the body mass index (BMI), HOMA-IR (D.R. Matthewsetal), calculated by means of the program HOMA Calculator Version 2.2 Diabetes Trials Unit University of Oxford (Great Britain). The diagnosis of DM was made according to the Order of the Ministry of Health of Ukraine № 1118dated 21.12.2012. The diagnosis "Type 2 diabetes mellitus" was verified on the basis of the "Unified Clinical Protocol of a Specialized Medical Aid: type 2 DM" (2012). Differential diagnostics of type 1 diabetes mellitus was made according to the Adapted clinical instructions based on the evidence "Type 1 Diabetes Mellitus"(2014).

Statistical analysis of the results obtained was carried out according to the type of the study conducted and types of numeric data obtained. Normality of distribution was checked by means of Shapiro-Wilk and Lilliefors tests, and the method of direct visual histogram assessment of own values distribution. Quantitative parameters having normal distribution are presented as a mean (M) ± standard deviation (S). Discrete values are presented in the form of absolute and relative frequencies (the percentage of observations to the total amount of the examined). To compare the data having normal character of distribution, parametric

Table I. Indicators of glycemia state and regulation of carbohydrate metabolism in patients suffering from chronic pancreatitis in the exacerbation stage with comorbid chronic obstructive pulmonary diseases and diabetes mellitus, in case of an isolated course of COPD and type 2 DM (M±S)

Indicator, un. of measur.	PHI, (n=32)	CP (1), (n=38)	CP + COPD (2), (n=35)	CP + COPD + DM (3), (n=37)	COPD (4), (n=32)	Type 2 DM (5), (n=34)
Glucose, mmol/l	3.95±0.18	5.72±0.26 *	5.85±0.15 *	12.67±0.45 */**/^	4.43±0.25 **/^/#	9.59±0.48 */**/^/#/##
Glucose p/p, mmol/l	6.86±0.20	8.19±0.25 *	9.28±0.27 */**	17.94±1.38 */**/^	7.15±0.21 **/^/#	15.80±1.33 */**/^/##
Insulin, mkOD/l	9.97±0.85	6.21±0.81 *	5.66±0.58 *	3.13±0.41 */**/^	9.52±0.73 **/^/#	28.35±3.08 */**/^/#/##
Glucagon, pg/ml	67.59±6.53	27.43±3.27 *	19.25±3.45 *	7.30±0.32 */**/^	59.88±6.21 **/^/#	118.67±12.11 */**/^/#/##
PP, pg/ml	75.47±8.28	22.53±6.25 *	16.90± 5.12 *	5.27±0.23 */**/^	70.61±8.18 **/^/#	96.22±8.16 **/^/#/##
Index Caro	0.40±0.01	0.92±0.01 *	1.03±0.02 */**	4.05±0.03 */**/^	0.47±0.01 */**/^/#	0.33±0.01 */**/^/#/##
HOMA-IR	1.21±0.20	0.84±0.05 *	0.77±0.04 *	0.51±0.01 */**/^	1.19±0.14 **/^/#	4.10±0.24 */**/^/#/##
HOMA S	82.80±6.27	119.53±7.25 *	130.30±8.12 *	195.62±15.47 */**/^	83.79±6.10 **/^/#	24.38±2.17 */**/^/#/##
HOMA B	181.42±30.27	62.90±5.21 *	56.42±4.48 *	8.11±1.13 */**/^	139.81±28.19 **/^/#	71.70±5.13 */#/##
Hb A1c, %	4.7±0.05	5.7±0.03 *	6.1±0.05 */**	6.8±0.07 */**/^	4.8±0.03 **/^/#	6.7±0.06 */**/^/##
BMI, kg/m^2	24.12±1.23	23.41±1.15	25.85±1.62	21.30± 1.06	26.45±1.21 #	29.15±1.28 */**/#

Notes: * – the difference is statistically significant ($p<0.05$) in comparison with the same indicator in practically healthy individuals;
** – the difference is statistically significant ($p<0.05$) in comparison with the indicator in patients with CP;
^ – the difference is statistically significant ($p<0.05$) in comparison with the indicator in patients with CP and COPD;
# – the difference is statistically significant ($p<0.05$) in comparison with the indicator in patients with CP, COPD and DM;
– the difference is statistically significant ($p<0.05$) in comparison with the indicator in patients with COPD.

tests with assessment of Student t-criterion or Fisher F-criterion were used. In case of abnormal distribution the median test and Mann-Whitney U test were used, the *Wilcoxon* Rank-Sum test was applied for multiple comparison (in case of examination of dependent groups). To assess the degree of dependence between the variables Pearson's correlation coefficient test for parametric distribution was used, and in case of distribution of parameters reliably different from that of the norm Spearman's correlation coefficient was used measuring the strength and direction of monotonic associations between two variables. To carry out statistical and graphic analysis of the results obtained the software packages Statisticafor Windows version 8.0 (StatSoftinc., USA), Microsoft Excel 2007 (Microsoft, USA) were applied.

RESULTS

Analysis of the results obtained showed a reliable increase of glucose content on an empty stomach in patients suffering from chronic pancreatitis with an isolated course 1.4 times ($p<0.05$) in comparison with PHI, and the level of glycemia increased when comorbid COPD joined chronic pancreatitis: 1.5 times in comparison with PHI ($p<0.05$) (Table I). At the same time, patients suffering from chronic pancreatitis with two comorbid diseases – COPD and DM – developed 3.2 times increased glucose concentration on an empty stomach in comparison with PHI ($p<0.05$). This parameter in the 1st and 2nd groups 2.1 and 2.2 times increased respectively ($p<0.05$). The state of glycemia on an empty stomach in patients from the 3rd group is similar to that with DM. Comparison of glucose content in the blood on an empty stomach in patients with type 2 diabetes mellitus with the parameter of this group found reliable 1.3 times difference ($p<0.05$), that appeared to be 2.4 times higher than that of PHI ($p<0.05$).

A reliably higher level of postprandial (after meals) glycemia was found in patients with chronic pancreatitis from the 1st group, that was 1.2 times higher than that of the control ($p<0.05$). At the same time, 1.4 times

increase of postprandial glycemia was registered in patients from the 2nd group ($p<0.05$), in the 3rd group –2.6 times as compared to (Table 1) the parameter of PHI ($p_{2,3}<0.05$),

Postprandial hyperglycemia was found in patients from the 5th group, that was 2.3 times higher than that of PHI ($p<0.05$), and that was 11.9 % lower than the parameters in the 3rd group ($p>0.05$).

The HbA1c content in the blood showed its reliable increase in patients from the 1, 2, 3 and 5 groups 1.2, 1.3, 1.4 and 1.4 times respectively in comparison with PHI ($p_{1,2,3,5}<0.05$) (Table I).

Examination of insulin content in the blood of patients from the 1st group found reliable hypoinsulinemia that was 1.6 times lower than that of PHI ($p<0.05$), though in patients from the 2nd and 3rd groups insulin content in the blood was reliably lower – 1.8 and 3.2 times respectively ($p_{2,3}<0.05$) (Table I).

Reliable hyperinsulinemia was found in patients from the 5th group, that was 2.8 times higher than that of PHI ($p<0.05$). Results of calculation of Caro index (glucose/insulin) showed that in patients from the 5th group this index was 1.2 times lower than that of PHI ($p<0.05$). At the same time, this index in patients from the 1, 2, 3 and 4 groups was 2.3, 2.6, 10.1 and 1.2 times higher respectively than that of PHI ($p_{1,2,3,4}<0.05$), with reliable difference between the groups ($p<0.05$). In patients from the 5th group of observation HOMA index of IR 3.4 times increased ($p<0.05$) HOMA%S index 3.5 times decreased ($p<0.05$) in comparison with indices of PHI. Assessment of the similar indices in the 1, 2 and 3 groups of patients found reliable decrease of HOMA IR index1.4, 1.6 and 2.4 times respectively ($p<0.05$) with underlying decrease of HOMA%S index 1.4, 1.6 and 2.3 times ($p<0.05$) in comparison with indices of PHI. Analysis of HOMA%B index in patients from the 1, 2 and 3 groups was found a decrease of 2.9, 3.2 and 22.6 times ($p<0.05$) in comparison with PHI.

The blood glucagon content in patients from the 1st group was 2.5 times lower in comparison with that of PHI ($p<0.05$), in patients from the 2 and 3 groups – 3.5 and 9.6 times respectively ($p_{2,3}<0.05$) (Table I). The pancreatic polypeptide content in the blood was 15 times lower in patients from the 3rd group in comparison with the reference value ($p<0.05$). At the same time, patients from 1 and 2 groups presented its reliable decrease 3.4 and 4.4 times respectively ($p<0.05$).

DISCUSSION

The goal of this study was to determine glycemic condition, regulation of carbohydrate metabolism, degree of insulin resistance in patients with chronic pancreatitis with its isolated course and with comorbid COPD and diabetes mellitus.

In situation of chronic pancreatitis developing occurrence of secondary diabetes mellitus is possible due to a decrease of the area or amount of functioning β-cells in the islet of Langerhans and absolute insulin insufficiency [9]. The case in question is type 3c diabetes mellitus occurring due to impairment in pancreatic endocrine function as a special nosologic and morphofunctional phenomenon [10]. The state of glycemia and regulation of carbohydrate metabolism in patients with chronic pancreatitis were assessed depending on comorbid pathology of COPD and DM.

Analysis of the laboratory findings concerning the level of postprandial glycemia shows, that it is indicative of the dependence of a degree of tolerance disorder to glucose on comorbid COPD and manifested DM.

Investigation of the laboratory results relating to HbA1c content in the blood serum as a marker of persistence and intensity of hyperglycemia showed its reliable increase in patients from the 1, 2, 3 and 5 groups, which confirms the role of chronic pancreatitis in the development of chronic postprandial hyperglycemia, advanced disorder to glucose tolerance, intensified glycosylation of transport proteins (hemoglobin), and further formation of DM.

The hyperinsulinemia was found in patients from the 5th group and was indicative of insulin resistance phenomenon. The insulin content in the blood of patients found reliable hypoinsulinemia that was which is indicative of absolutely insufficient insulin secretion by β-cells in the islet of Langerhans and the role of chronic inflammatory process in the pancreas promoting development of DM. Moreover, comorbid COPD promotes DM development in patients with chronic pancreatitis, since it intensifies inflammation, oxidative and nitric stress under conditions of hypoxia with intensified damage of the acinar epithelium of the pancreas and β- cells in the islet of Langerhans, development of hypoinsulinemia and hyperglycemia, which was reported in our previous studies [5].

The valuation of Caro index confirms insulin resistance syndrome available in patients with type 2 DM. Nevertheless, this index in patients with chronic pancreatitis, COPD and type 3c diabetes mellitus was higher respectively than that of practically healthy individuals, which confirms absolute insufficiency of insulin secretion by β- cells in the islet of Langerhans.

Calculation of insulin resistance index by means of HOMA2 model found deep insulin resistance available with underlying decreased sensitivity of the peripheral tissues to insulin in patients with type 2 diabetes mellitus. Assessment of the similar indices in the patients with

chronic pancreatitis, COPD and T3cDM was found reliable decrease of HOMA IR index with important decrease of HOMA%S index and investigation of HOMA%B index in these patients found a decreased portion of functioning β-cells in the parenchyma of the pancreas. It might occur due to chronic inflammation and scarring of the pancreas. This decrease was found to be reliable, which explains a cause of DM formation in the triple pathology group.

Correlation analysis of patients with chronic pancreatitis and comorbid COPD and DM found certain correlation between fecal elastase-1 content and HOMA%B value ($r=0.58$, $p<0.05$), strong correlation between insulin content on an empty stomach and HOMA%B value ($r=0.72$, $p<0.05$).

Absolute insulin insufficiency is found in two pathological conditions – type 1 DM and T3cDMc. To solve the issue concerning the type of DM in patients with chronic pancreatitis the content of glucagon and pancreatic polypeptide was determined in the blood. Thus blood glucagon content in patients with first three groups was lower than in PHI, which is indicative of an insufficient glucagon secretion by α-cells of the pancreas and confirms the role of chronic pancreatitis in the development of T3cDM. One more important factor which is indicative of the development of T3cDM in patients with comorbid pathology of chronic pancreatitis and COPD is pancreatic polypeptide content in the blood. The PP content in the blood was lower in every group, which includes patients with chronic pancreatitis. It should be noted that in physiological concentrations PP acts as cholecystokinin antagonist, inhibits pancreatic juice secretion and relaxes non-striated muscles of sphincters in the gastrointestinal tract, hepatic-biliary system etc. [14]. According to the data found in scientific publications PP level in the blood is lowered in patients with chronic pancreatitis, which is confirmed by the results of our studies [10, 15, 17, 21]. Therefore, pathogenic loss of β-cells mass in patients with chronic pancreatitis stipulates endocrine insufficiency of the pancreas. Meanwhile, contrary to type 1 diabetes mellitus in addition to decreased insulin secretion the secretion of glucagon and pancreatic polypeptide decreases as well, which can result in the development of the so-called "brittle disease" (fragile) with considerable variations of glycemia and increasing risks of uncontrolled hypoglycemia episodes [11, 18-19, 23].

CONCLUSIONS

1. Chronic pancreatitis in its exacerbation stage without comorbid pathology is associated with reliable postprandial hyperglycemia (1.2 times), an increased content of glycated hemoglobin (1.2 times), hypoinsulinemia on an empty stomach (1.6 times), a decreased content of glucagon in the blood (2.5 times) and pancreatic polypeptide (3.4 times), a considerable decrease of HOMAB value (2.9 times) ($p<0.05$), which is indicative of initial signs of carbohydrate metabolism dysfunction.

2. A comorbid course of chronic pancreatitis with exacerbated COPD (2-degrees D) is associated with more intensive disturbances in carbohydrate metabolism regulation and glycemia parameters in comparison with an isolated course of chronic pancreatitis: a higher ($p<0.05$) degree of postprandial hyperglycemia (1.4 times), an increased content of glycated hemoglobin (1.3 times), hypoinsulinemia (1.8 times), a decreased content of glucagon in the blood (3.5 times) and pancreatic polypeptide (4.4 times), decrease of HOMAB value (3.2 times) ($p<0.05$), which is indicative of COPD role in additional damage of α-cells and β-cells in the islet of Langerhans with underlying inflammatory process in the pancreas due to hypoxia, activation of oxidative stress, inflammation, cytokine imbalance and fibrosis of the pancreatic tissue.

3. In case comorbidity includes three components such as chronic pancreatitis, COPD (2-3 degree D) and diabetes mellitus, the most unfavorable glycemic profile is found which is indicative of carbohydrate metabolism decompensation: hyperglycemia on an empty stomach (3.2 times), postprandial hyperglycemia (2.6 times), an increased content of glycated hemoglobin (1.4 times), hypoinsulinemia on an empty stomach (3.2 times), a decreased content of glucagon in the blood (3.5 times) and pancreatic polypeptide (4.4 times), a considerable decrease of HOMA B value (22.6 times) ($p<0.05$), which correlates in the interdependence of the average strength with the marker of exocrine pancreatic insufficiency – fecal elastase-1 content ($r=0.58$, $p<0.05$). It is indicative of the formation of T3c DM – pancreatogenic diabetes mellitus with total synthesis insufficiency of insulin, glucagon and pancreatic polypeptide with a strong correlation between fasting insulin level and HOMA% B ($r=0.72$, $p<0.05$). The signs of insulin resistance with chronic pancreatitis and comorbid COPD and T3cDM were not found.

4. Patients with type 2 diabetes mellitus with an isolated course presented an excessive body weight (BMI 1.2 times higher), hyperglycemia on an empty stomach (2.4 times) and postprandial hyperglycemia (2.3 times), an increased content of glycated hemoglobin (1.4 times), hyperinsulinemia on an empty stomach (2.8 times), an increased content of glucagon in the blood (1.8 times) and pancreatic polypeptide (1.3 times), decreased Caro index (1.2 times), increased HOMAIR (3.4 times), decreased HOMAS index (3.5 times) ($p<0.05$), which is indicative of considerable insulin resistance available specific for insulin dependent type 2 diabetes mellitus.

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ORIGINAL ARTICLE

PATIENT-CENTERED CARE AND SELF-MANAGEMENT: OPINION OF MILITARY PERSONNEL WITH CORONARY ARTERY DISEASE

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ABSTRACT

The aim: To determin attitude of military personnel with coronary artery disease to implementation of the principles of patient- centered care and self-assessment of adherence to treatment

Materials and methods: 72 military personnel (male aged 30–58 years) with coronary artery disease, who visited the general practitioners at the Outpatient Care Clinic of the National Military Medical Clinical «Main Military Clinical Hospital» were interviewed anonymously, using the specially designed questionnaire. The patients were divided into two groups: the 1st group with individuals of 49 years old and younger (39 military personnel, mean age 42,8±5,0) and the 2nd group who is 50 years and older (33 military personnel, mean age 53,2±2,4 years).

Results: The results of a sociological survey showed that the majority of military personnel with coronary artery disease believe that doctors do not always provide them with enough information about their health (61,1%) and they do not always provide emotional support to solve their health problems (66,7 %). It has been indicated a mismatch between patients' willingness to participate in shared decision-making regarding a treatment (84,7 % of them) and adherence to treatment – 55,6% of surveyed patients rated adherence to treatment by three points or less on a five-point scale. There is no statistically significant difference between military personnel of the 1st and 2nd groups.

Conclusions: The results of the study showed the interest and readiness of surveyed military personnel with coronary artery disease to implementation of the principles of patient-centered medical care.

KEY WORDS: military personnel, coronary artery disease, patient- centered care

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INTRODUCTION

The patient-centered care model has become a key element of the health care quality and provides that care should meet the individual preferences, needs, and values of patients, and take into account the patient's wishes when making clinical decisions about medical services [1-3]. This approach shifts the focus from the traditional model, in which a physician plays the primary decision-making role, to one that supports a partnership among practitioners and patients [2]. Modern scientific publications use the term «person-centered care», «person-directed care», «person-focused care», which includes, in addition to medical, other human needs. WHO recommends introducing people-centered health care as a priority in the development of health systems in the 21st century [1]. The improving of patient-centered care requires addressing many issues, including the evaluation of the effectiveness of their use in clinical practice – both from the standpoint of patients and health personals [4, 5]. It should be noted that there is no generally accepted approach to evaluating the effectiveness of patient-centered care. Researchers use surveys of patients, surveys of physicians and analysis of clinical trial results. A large number of questionnaires have been proposed to assess patient-centered care [4]. The CARE Measure (The Consultation and Relational Empathy Measure) is a simple, rigorously tested questionnaire to assess patient-centered care, which is widely used in clinical trials. This questionnaire provides questions to determing the use of the shared decision-making approach, and the level of empathy. [4]. An important component of person-centered care implementation is patient`s educating, engaging in self management support of health, and increasing adherence to physician`s prescriptions [1]. The results of a study on the use of patient-centered education on adherence to the treatment regimen in patients with coronary artery disease (CAD), published in 2022, confirmed the positive effect to adherence. [6]. This aspect is also important for improving medical care

for military personnel of the Armed Forces of Ukraine with CAD. To optimize the organization of medical care, it is important to regularly analyze the use of the basic principles of patient-centered care, taking into account the results of a survey of military personnel.

THE AIM

To determin attitude of military personnel with CAD to implementation of the principles of patient-centered medical care and self-assessment of adherence to treatment.

MATERIALS AND METHODS

We performed a poll of 72 military personnel (male aged 30–58 years, average age 47,6±6,5 years) with coronary artery disease, who visited the general practitioners at the Outpatient Care Clinic of the National Military Medical Clinical «Main Military Clinical Hospital». When the size of the research sample was determined, we used generally accepted approaches and calculations taking into account the general population of military personnel who were examined during the period of the research. The following inclusion criteria apply: military personnel, men, 25 to 60 years of age, patients with diagnosis of coronary artery disease who present with stable (nonemergent), informed consent. Exclusion criteria: patients being evaluated for other cardiac diseases (e.g., valvular disease, etiology of cardiomyopathy). To study the age-related characteristics, patients were divided into two groups: the 1st group with individuals of 49 years old and younger (39 military personnel, mean age 42,8±5,0) and the 2nd group who is 50 years and older (33 military personnel, mean age 53,2±2,4 years). Surveyed military personnel with CAD had 2 or more chronic diseases: 2 diseases – 9 of 72 surveyed (12,5 %); 3-5 diseases – 32 of 72 surveyed (44,4 %), 6 or more diseases – 31 of 72 surveyed (43,1 %). Surveyed military personnel with CAD received routine medicines treatment. Number of pills for daily intake: 2-5 pills– 19 of the 72 surveyed (26,4 %), 6-10 pills –33 of the 72 surveyed (45,8 %), 10 pills or more – 20 of the 72 surveyed (27,8 %).

The following methods were used to achieve the aim: sociological, statistical, systematic approach and analysis. The research material is a questionnaire with a total number of 72 items. The questionnaire was designed by the authors to determine attitude of military personnel with CAD to implementation of the principles of patient-centered medical care and self-assessment of comply physician`s recommendations. The survey questionnaire included two blocks of questions. The first block included questions about patient's assessment of doctor's use of the shared decision-making approach, determination of the level of empathy, provide information that corresponds to the main provisions of the CARE questionnaire [4]. The second block of questions included questions to assess the readiness of military personnel with CAD to perform self-monitoring of health and participation in the shared decision-making for treatment; self-assessment of adherence to physician's recommendations on non-drug and drug treatment and factors influencing on adherence to treatment.

Statistical data analysis was performed by the use of standard statistical package (Statistica v. 6.0) and Microsoft Excel 2007. Categorical data were presented as absolute and relative (%) frequency. To enable comparisons, we calculated the mean value (M), and the standard error of the mean (m). The statistical sample was checked for distribution according to the normal law. Student's t-test was used to compare the mean of a data for the two groups. The results were considered significant at $p < 0,05$.

RESULTS

Patient-centered care is based on partnership among health professionals and patients, so the information provided to a patient is a very important component, as well as the involvement in the shared decision-making for the treatment.

The results of a sociological survey showed that the majority of military personnel with CAD – 44 of 72 (61,1%) believe that doctors do not always provide them with enough information about their health and course of the disease: 61,6 ± 7,8% of the 1st group, and 60,6 ± 8,5% of the 2nd group ($p = 0,93$); 21 of 72 respondents (29,2%) answered that doctors provide sufficient information: 20,5 ± 6.5% of the 1st group, and 33,3 ± 8,2% of the 2nd group ($p = 0,23$); 7 of 72 respondents (9,7%) believe that they are provided with insufficient information. The shared decision-making requires discussion of many issues with a patient. To the question « Does the doctor agree with you your treatment plan?» – «Yes» answered 43 of the 72 surveyed military personnel (59,7%): 66,7 ± 7,5% of the 1st group, and 51,5 ± 8,7% of the 2nd group ($p = 0,20$), «Not always» – 22 of 72 patients (30,6%): 23.0 ± 6,7% of the 1st group, 39,4 ± 8,5% of the 2nd group ($p = 0,14$); «No» – 7 of 72 patients (9,7%).

The next questions of the questionnaire were to discuss the provision of information by doctors on the appointment of medical treatment. To the question «Does the doctor discuss with you for what purpose he prescribes medication?» – «Yes» answered 60 of the

72 surveyed military personnel (83,3%): 84,6 ± 5,8 of the 1st group, 81,8 ± 6,7% of the 2nd group (p = 0,75), «No always « – 10 of 72 patients (13,9%); «No» – 2 of 72 patients (2,8%).

To the question « Does the doctor discuss with you the side effects of the prescribed drugs?» – «Yes» answered 20 of the 72 surveyed military personnel (27,8%): 23,1±6,7% of the 1st group, 33,3±8,2% of the 2nd group (p=0,35), «No always « – 37 of the 72 patients (51,4 %): 56,4±7,9% of the 1st group, 45,5±8,7% of the 2nd group (p=0,36); «No» – 15 of the 72 respondents (20,8%). To the question about the doctor's explanation of the medication regimen – «Yes» answered 37 of the 72 respondents (51,4 %): 46,2±8,0% of the 1st group, 57,6±8,6% of the 2nd group (p=0,34), «No always « – 28 of the 72 patients (38,9 %): 41,0±7,9 of the 1st group, 36,3±8,4% of the 2nd group (p=0,69); «No» – 7 of the 72 respondents (9,7%).

To the question «Does the doctor discuss with you measures to ensure that you do not forget to take drugs daily?"– «Yes» answered 20 of the 72 surveyed military personnel with CAD (27,8 %), «No always « – 48 of the 72 patients (66,7 %): 82,1±6,1% of the 1st group, 48,5±8,7%, of the 2nd group (p=0,03); «No» – 4 of the 72 respondents (5,6%). Thus, the interviewed military personnel with CAD need more information about drug therapy.

Patient-centered care involves the active participation of an informed patient in the process of self-monitoring. To the question «Does your doctor provide you with information on participation in self-monitoring of your health, and the results of treatment (measuring blood pressure, heart rate, angina attacks, etc.)?» – «Yes» answered 30 of the 72 respondents (41,7 %): 43,6±7,9% of the Ist group, 39,4±8,5% of the 2nd group (p=0,72), «No always « – 39 of the 72 patients (54,2 %): 56,4±7,9 of the 1st group, 51,5±8,7% of the 2nd group (p=0,68); «No» – 3 of the 72 (4,2%).

An important part of patient-centered care is the emotional support of the patient by health professionals. To the question about the doctor's emotional support in solving patient health problems the majority of military personnel with CAD answered «Not always « – 48 of the 72 patients (66,7 %): 71,8± 7,2% of the 1st group, 60,6±8,5% of the 2nd group (p=0,32); «Yes» – answered 14 of the 72 (19,4 %); «No» – 10 of the 72 patients (13,9%).

The health professionals – patients partnership is one of the basic principles of patient-centered care, which involves an active participation of a patient. To the question of the questionnaire "As for you, does the patient have the right to participate in deciding on the appointment of examinations and treatment? – «Yes» answered 64 of the 72 respondents (88,9%): 92,3±4,3% of the 1st group, 87,9±5,7% of the 2nd group (p=0,54), «I can't decide» – 7 of 72 patients (9,7 %). Almost all surveyed military personnel with CAD (64 of the 72 – 90,3%) answered that they need to discuss a treatment plan with their doctor in more detail and be involved in the decision: 92,3 ± 4,0% of the 1st group, 84,8±6,3% of the 2nd group (p=0,27).

The logical question was «Are you ready to take measures to self-monitor your health and participate in the shared decision-making?» «Yes» answered 61 of the 72 surveyed military personnel with CAD (84,7 %): 84,6 ±5,8% of the 1st group, 84,8±6,3 % of the 2nd group (p=0,98).

The patient-centered care, shared decision-making involves a responsible attitude of the patient and participation in the implementation of the decision. This aspect is closely related to the patient's adherence to follow the doctor's recommendations. To the question «Assess your compliance with the doctor's recommendations on healthy diet on a five-point scale» – by 5 points – no one rated themselves, by 4 points – 15 of the 72 respondents (20,8 %): 28,2±7,2% military personnel of the 1st group and 12,1±5,7% of the 2nd group (p=0,09); by 3 points or less – 64 of 72 patients (71,9%): 71,8 ± 7.2% of the 1st group, 87,9 ± 5.7% of the 2nd group (p = 0,09). To the question «Assess your compliance with the doctor's recommendations on physical activity (walking, exercise, etc.) on a five-point scale»– by 5 points – no one rated themselves, by 4 points – 22 of the 72 surveyed military personnel with CAD (30,6 %): 30,8±7,4% of the 1st group, 30,3±8,0% of the 2nd group, p=0,96, by 3 points or less – 50 of 72 patients (69,4 %): 69,2±7,4% of the 1st group, 69,7±8,0% of the 2nd group, p=0,96. To the question «Assess your compliance with the doctor's recommendations for medication on a five-point scale» – by 5 points – no one rated themselves, by 4 points – 32 of the 72 surveyed military personnel with CAD (44,4 %): 43,6 ±7,9 % of the 1st group, 45,5 ± 8,7 of the 2nd group (p=0,87), by 3 points or less – 40 of 72 patients (55,6%): 56,4 ± 7,9% of the 1st group, 54,5±8,7% of the 2nd group (p=0,87). Thus, the results of the study showed a lack of adherence to non-drug and drug treatment in majority of surveyed military personnel with CAD.

The questionnaire included questions to determine the factors that make it difficult to follow the doctor's recommendations on medication. The survey results are listed in table I.

It was found that the main factor patients called – «I forget to take medication» and «a large number of drugs for daily use» (Table I). There is no statistically significant difference between the military personnel of the 1st and 2nd groups.

Table I. Factors affecting adherence to drug treatment

Factors	All patients (n=72)		1st group (n=39)		2nd group (n=33)		p I-II
	number of patients	% (P ± m)	number of patients	% (P ± m)	number of patients	% (P ±m)	
A large number of drugs for daily use	35	48,6± 5,9	18	46,2± 8,0	17	51,5± 8,6	0,66
I forget to take medication	51	70,8± 5,4	30	76,9± 6,7	21	63,6± 8,4	0,23
Distrust of the positive effects of drugs on health	23	31,9± 5,5	14	35,9± 7,7	9	27,3± 7,8	0,44
Financial constraints	26	36,1± 5,7	9	23,1± 6,7	17	51,5± 8,7	0,01
I'm afraid of side effects	21	29,2± 5,4	11	28,2± 7,2	10	30,3± 8,0	0,85

DISCUSSION

CAD is a disease that requires long-term comprehensive treatment. Improving the effectiveness of a treatment for patients with chronic diseases in curent conditions is associated with implementation of patient- centered care [1, 3, 7]. Recent scientific publications identified eight principles of patient-centered care: respect for patient's values, preferences, and expressed needs, provision of information and education, emotional support to relieve fear and anxiety, involvement of family and friends, physical comfort and symptoms relief, continuity and secure transition between health care settings, coordination of care, access to care [2, 3]. One of the main elements of patient-centered care is to treat a patient with dignity and respect – as a person, not as a clinical case – a combination of diseases or symptoms [2]. This is an important component of shared decision-making, involving a patient in the treatment process as a partner, not just as a recipient of care [8]. The implementation of this approach requires appropriate training for both physicians and patients. At the current stage of improving medical care for military personnel in Ukraine, the introduction of a patient-centered approach is important. The results of our study showed that surveyed military personnel with CAD need more information and discussion with the physicians about their health state, the course of the disease, prescribed medication, and providing information on patient participation in self-monitoring. The obtained results should be taken into account for more effective implementation of the patient-centered model of health care for military personnel with CAD, in particular for the training of healthcare profecionals on these issues.

Emotional support – an important component of patient-centered care, which aims to improve the emotional state of a patient, relieve fear and anxiety [5]. Surveys conducted by S.B Frampton et all. [9] found that most patients choose empathy, respect, and kindness as critical to medical counseling. Empathy is the ability to listen and hear, empathize, and emotionally support a person receiving medical care. In addition, patients who have a good relationship with the healthcare professionals tend to indicate satisfaction with the care provided and have a better adherence to treatment [10]. The results of our study justify the need to improve physicians' communication skills and empathy skills, because only 19,4% of surveyed military personnel with CAD affirmative answered to the question «Do you receive emotional support from a doctor to solve health problems?»

Partnerships and the shared decision-making are not always unambiguously perceived by patients. Often patients want to receive information, but do not want to be responsible for the shared decision-making. Therefore patients education should be aimed at involving them in self-monitoring and self-care [1]. The results of our study indicate a mismatch between patients' willingness to participate in shared decision-making regarding treatment and adherence to treatment. Insufficient adherence to treatment is a typical problem in the treatment of patients with chronic diseases, including CAD, and has a negative impact on the prognosis [11]. Factors related to adherence to a treatment include patient's knowledge, physician`s communication skills, and more [12]. According to the results of our study, the main factor that makes it difficult to follow the adherence to treatment, surveyed military personnel with CAD called – «forget» (70,8%). This factor was identified as leading in the study R Khatib et. al. [11] – 84.9% of CAD patients indicated forgetfulness as a reason of non-adherence to physician`s recommendations. In our study, half of the surveyed military personnel with CAD identified a large number of drugs

for daily use as a factor that makes it difficult to follow the doctor's recommendations. This fact must be taken into account, in particularly, to consider the possibility of prescribing polypills, which is recommended in modern clinical guidelines for the management of patients with CAD [13]. Among the factors that make adherence to treatment difficult, almost 30% of surveyed military personnel with CAD (Table I) identified distrust in the positive effects of drugs and fear of side effects, which may be due to lack of knowledge. Studies conducted by M. Saki et. al. [6] show that patient-centered education is effective in improving adherence to the treatment regimen in patients with CAD. Recommendations for patients education and their active involvement in the process of self-monitoring and self-care are considered at the current stage as an important component of clinical guidelines. Thus, in «2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure» [14] includes a separate section «Patient education, self-care and lifestyle advice», considers all aspects of patient participation – self-support, monitoring, patient actions in changing clinical manifestations. This approach is fully consistent with the introduction of the principles of patient-centered care.

CONCLUSIONS

1. It has been defined that the majority of military personnel with CAD believe that doctors do not always provide them with enough information about their health – 61,6 ± 7,8% of the 1st group, and 60,6 ± 8,5% of the 2nd group (p = 0,93) and they do not always provide emotional support to solve their health problems – 71,8± 7,2% of the 1st group, 60,6±8,5% of the 2nd group (p=0,32).
2. The results of the study showed the interest and readiness of surveyed military personnel with CAD to implementation of the principles of patient-centered medical care. Almost all surveyed military personnel with CAD (90,3%) answered that they need to discuss a treatment plan with their doctor in more detail and be involved in the shared decision-making: 92,3 ± 4,0% of the 1st group, 84,8±6,3% of the 2nd group (p=0,27).
3. It has been determined a mismatch between the willingness of patients to participate in shared decision-making of treatment (84,6 ±5,8% of the 1st group, 84,8±6,3 % of the 2nd group (p=0,98)) and adherence to treatment – most patients (56,4 ± 7,9% of the 1st group, 54,5±8,7% of the 2nd group, p=0,87) rated adherence to treatment on three points or less on a five-point scale.
4. The results of our study justify the need to improve approaches to raising education, involving patients with CAD to shared decision-making, increasing adherence to treatment and improving physicians communication skills as part of the introduction of patient-centered care.

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ORIGINAL ARTICLE

ASSESSMENT OF CARDIOPROTECTIVE EFFECT OF NECROSTATIN-1 STABLE IN A MICE MODEL OF ACUTE DOXORUBICIN-INDUCED CARDIOTOXICITY

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ABSTRACT

The aim: To evaluate the effect of Necrostatin-1s (Nec-1s), an inhibitor of necroptosis, on acute Dox-induced cardiotoxicity in a mice model.

Materials and methods: Fifteen male mice were used. The animals were allocated into three groups. On the third day of the experiment, a single intraperitoneal dose of 20 mg/kg Dox was used to induce cardiotoxicity. Mice in the control group were given vehicle (DMSO) intraperitoneally, whereas mice in the third group were given 5 mg/kg Nec-1s two days before Dox treatment and continued for a total of five days. Animals were euthanized at the conclusion of the research. ELISA was used to assess the following parameters: cTnI, TNF-α, IL-1β, GPX-4, and Hmox-1. The expression of TNF-R1 and phosphorylated NF-κβ p65 was measured using immunohistochemistry. In addition, a histopathologic evaluation of the cardiac lesions was conducted.

Results: Our results showed that Dox treatment substantially elevated serum cTnI levels, increased tissue inflammatory biomarkers (TNF-α, IL-1β, phospho NF-κβ p65 and TNF-R1), and reduced tissue antioxidant enzymes (GPX-4, Hmox-1). A histopathological analysis showed pronounced necrosis and vacuolization. These results were drastically changed by pretreatment with Nec-1s, with serum cTnI levels in this group being much lower than in the Dox group. In addition to a significant decrease in inflammatory markers, antioxidant enzymes were partially recovered. Moreover, there was preservation of the cardiac morphology to a level that was roughly normal.

Conclusions: Our findings demonstrate that pretreatment with Nec-1s protected against acute Dox-induced cardiotoxicity. This cardioprotective effect was mainly due to amelioration of inflammation that reflected by inhibition of NF-κβ/TNF-α/TNF-R1 pathway, with partial restoration of antioxidant enzymes, GPX-4 and Hmox1.

KEY WORDS: doxorubicin, cardiotoxicity, necroptosis, Necrostatin-1s

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INTRODUCTION

Doxorubicin (Dox) is a powerful and efficient antineoplastic drug that is commonly used to treat a variety of cancers. Dox's therapeutic application is restricted due to its dose-dependent cardiotoxic impact [1]. While the precise mechanism of the cardiotoxicity is still unknown, it has been observed that increase reactive oxygen species generation with defective antioxidant systems, increase cytokines production, as well as mitochondrial dysfunction play an important role during cardiotoxicity [2]. In recent years, molecular mechanisms of Dox-induced cardiotoxicity were re-investigated where novel forms of cell death were blamed as major participants, particularly, autophagy, ferroptosis, and necroptosis [3]. Necroptosis is a controlled kind of necrosis that includes the release of cytokines that indicate cell death [4]. The binding of TNF–α to tumor necrosis factor receptor–1 (TNF-R1) stimulates complex–I formation. This complex consists of Adaptor TNF receptor 1–associated death–domain (TRADD), receptor–interacting protein-1 kinase (RIPK-1), TNF receptor–associated factor-2 (TRAF–2), inhibitor of apoptosis proteins cIAP–1 and cIAP–2, which can activate NF–κB [5]. Phosphorylation of RIPK-1 recruits and activates RIPK-3, resulting in the formation of the necroptosome.The necroptosome then activates the mixed lineage kinase domain–like protein (MLKL–1), causing the plasma membrane to rupture and release organelles and inflammatory substances, prompting an immunological response and cell death [6]. Several small molecules known as Necrostatin like Necrostatin-1 and Necrostatin-1s (Nec-1s) could achieve Necroptosis inhibition. While necrostatin-1 has been utilized in multiple trials and has been found to protect

against several necroptosis-related disorders, it has the following drawbacks: Necrostatin–1 has an off–target effect on indolamine 2, 3–dioxygenase (IDO); its half-life is only approximately one hour [7]. Necrostatin-1s (Nec-1s) also called 7-Cl-O-Nec-1, is a more potent and selective RIPK1 inhibitor than necrostatin-1 since it does not inhibit IDO. It is approximately two folds more effective than Necrostatin-1 [8]. Additionally, Nec-1s protects against TNF-induced systemic inflammatory response syndrome without causing a paradoxical sensitizing effect, so having a more safety profile than Necrostatin-1 [7]. In a mice model of cardiac ischemic reperfusion injury, Nec-1s at a dose of 3-6 mg/kg showed to decrease myocardial necroptosis and ameliorate ischemic reperfusion injury [9]. Similarly, it was reported that treatment with Nec-1s could provide protection in hepatic ischemic reperfusion injury. It has also shown to protect against acute kidney injury in a recent preclinical study [10]. As the preventive benefits of Nec-1s have been shown in several pathological processes, the purpose of this investigation was to examine the protective effects of Nec-1s on acute Dox-induced cardiotoxicity.

MATERIALS AND METHODS

Fifteen male mice (25–30 g) were used in this study. They were purchased from the Faculty of Science/ University of Kufa. During the study, the rules for the protection of animal rights were carefully followed (ethical approval no. 7924 in 30/3/2022). The animals were allocated into three groups (five mice per group) as follows:

1. Control group: received an intraperitoneal (I.P) injection of 0.3 ml dimethyl sulfoxide for five days
2. Dox group: received 20 mg/kg Dox I.P injection as a single dose on the 3rd day of the experiment
3. Dox plus Nec-1s group: received 20 mg/kg Dox, I.P as a single dose on the 3rd day of the experiment plus 5 mg/kg Nec-1s, I.P, daily for 5 days, starting two days before Dox injection).

After five days, in all groups, mice were anesthetized with 80 mg/kg ketamine+10 mg/kg xylazine I.P. For biochemical investigation, blood was taken through a thoracotomy and direct heart puncture. The animals were subsequently anesthetized and euthanized.

The hearts were removed, weighed, and then divided into two parts after being rinsed twice with an ice-cold buffer solution. For the tissue homogenization technique, basal portions were used. The homogenates were centrifuged at 4°C for 10 minutes at 10000 rpm. Supernatants were utilized to determine tissue markers. For histopathological and immunohistochemistry analysis, the apical portions were fixed in 10% formalin. Five µm thick sections of tissues were stained with hematoxylin-eosin. Finally, samples were examined under the light microscope (Olympus, Japan), and micrographs were taken.

ENZYME-LINKED IMMUNOASSAY

Cardiac troponin I (cTnI) was measured in serum using a mouse ELISA kit as a marker of acute myocardial damage (ELK Biotechnology, China). The cardiac tissue homogenate was utilized to detect inflammatory markers (TNF-α and IL-1β), anti-oxidant enzymes (glutathione peroxidase-4 (GPX-4) and heme oxygenase-1 (Hmox-1)), using their respective ELISA kits (ELK Biotechnology, China).

HISTOPATHOLOGICAL ANALYSIS

According to Bellingham and colleagues' description [11], the following scale was used to grade the histopathological changes: (I) swelling of the myocardial fibers and interstitial edema (1+) (II) myocardial cytoplasmic/perinuclear vacuolization (2+), (III) myocardial fiber necrosis (3+), and (0) when no damage observed in the heart.

IMMUNOHISTOCHEMISTRY STAINING

The immunohistochemistry staining method was conducted according to manufacturer instructions (Sunlong Biotech Co. LTD). The sections were incubated in 3% hydrogen peroxide (H_2O_2) for 5-10 minutes then washed in buffer saline. Then, incubated with primary antibodies (a rabbit anti-TNF-R1 (1:300), rabbit anti-phospho NF-κβ-65 (1:300), ELK Biotechnology, China,) overnight at 4°C. After rinsing them with buffer saline, sections were incubated with anti-mouse/rabbit antibodies as secondary antibodies for 30 minutes and were placed in avidin-peroxidase complex solution for 30 minutes. Peroxidase activity was revealed by dipping the sections for five minutes in a mixture of DAB and H_2O_2. The sections were slide-mounted, dehydrated, and cover-slipped. The level of expression was graded into four grades: 0/1/2/3 dependent on staining intensity, where 0 indicates no stain or negative, 1 referred to weak stain, 2 referred to moderate intensity stain, and 3 referred to strong intensity stain [12].

STATISTICAL ANALYSIS

Statistical data were computed using Statistical Package for the Social Sciences (SPSS) software. The data were presented as means ± standard deviation. Between

groups analysis was conducted using one-way analysis of variance (ANOVA) and post-hoc LSD testing. For histopathological and immunohistochemistry scores, Kruskall-Wallis test with pairwise analysis was used P <0.05 was deemed statistically significant.

RESULTS

EFFECTS ON SERUM cTnI

Dox treatment significantly increased serum cTnI levels (P=0.001). Compared to Dox group, Nec-1s pretreatment significantly reduced serum cTnI concentration (P=0.001) and virtually restored it to control level (P=0.711), with no significant difference between the control and Nec-1s groups (Fig. 1A).

EFFECTS ON CARDIAC TISSUE INFLAMMATORY MARKERS (TNF-α, IL-1β)

Cardiac TNF-α and IL-1β levels were both considerably higher in the Dox group compared to the control group (P=0.001). Their levels were significantly reduced by Nec-1s pretreatment compared to the Dox group (both P=0.001). TNF-α levels, on the other hand, remained considerably elevated in the Nec-1s pretreated group in comparison to the control group (P=0.025); nevertheless, IL-1β levels did not show any significant variation between control and Nec-1s pretreated groups (P=0.216) (Fig. 1B, 1C).

EFFECTS ON CARDIAC TISSUE ANTI-OXIDANT PARAMETERS (GPX-4 AND Hmox-1)

As compared to the control group, cardiac GPX-4, and Hmox-1 levels were found to be considerably lower in the Dox group (P=0.02, 0.001 respectively). As compared to the Dox group, the Nec-1s administration resulted in a considerable elevation of Hmox-1 concentrations (P=0.001). On the other hand, although the GPX-4 level was upregulated by Nec-1s administration, this effect was not statistically significant (P=0.067) (Fig. 1D, 1E).

EFFECT ON TNF-R1 EXPRESSION

Dox administration caused significant upregulation of TNF-R1 expression when compared to control, P=0.006. This was manifested by an increase in the intensity of immunostaining. Treatment with Nec-1s caused a significant reduction in the intensity of TNF-R1 expression compared to Dox group, P=0.022, with no significant difference observed between Nec-1s and control groups (P=0.647), (Fig. 2, Fig. 5).

EFFECT ON PHOSPHO-NF-KB P65 EXPRESSION

As expressed by the intensity of immunostaining, Dox administration caused significant upregulation of p-NF-κβ p65 expression when compared to control, P=0.003. While pretreatment with Nec-1s caused a significant reduction in the intensity of p-NF-κβ p65 expression compared to Dox group (P=0.036). Additionally, no significant difference was observed between Nec-1s and control groups (P=0.403), (Fig. 3, Fig.6).

EFFECTS ON THE HEART'S HISTOPATHOLOGY

The histology of the heart tissue in the control group was consistent with normal morphology (Fig.4). Myocardial lesions were significantly worse in the Dox group than in the control group (P=0.005). Cytoplasmic vacuolization, multifocal congestion, and necrosis were the obvious signs of these lesions. The lesions in the Nec-1s pretreated group, on the other hand, were modest in intensity and exhibited minor interstitial edema with no evident cytoplasmic vacuolization or myofibrillar necrosis. This was considerably lower in comparison to the Dox group (P=0.02). There were no significant differences in severity score between Nec-1s pretreated group and control one (P=0.619).

DISCUSSION

Cardiotoxicity is the most significant and severe adverse effect of Dox. Many mechanisms, including oxidative stress, autophagy, mitochondrial dysfunction, apoptosis, and necroptosis pathways, contribute to doxorubicin-induced cardiotoxicity [3]. In the current study, a single intraperitoneal injection of 20 mg/kg of Dox was able to induce cardiotoxicity, as evidenced by increased serum cTnI and histopathological changes, elevated inflammatory parameters (TNF-α, IL-1β, TNF-R1, and p-NF-κβ65) and decreased anti-oxidant enzymes (GPX-4, Hmox-1) cTnI is one of the most reliable and extensively used biomarkers for evaluating cardiotoxicity [13]. In our study, Dox treatment caused cardiomyocytes damage, as shown by a significant increase in the serum level of cTnI compared to the control group. This is consistent with a number of experimental investigations describing the reaction of cTnI to Dox [14, 15]. In the current study, Nec-1s was able to reduce serum cTnI levels to a level close to normal. This agent's efficacy and safety have been examined in the ischemia-reperfusion I/R model of cardiac damage. This research demonstrated that Nec-1s protected isolated Langendorff/perfused rat hearts from I/R damage by reducing infarct size and

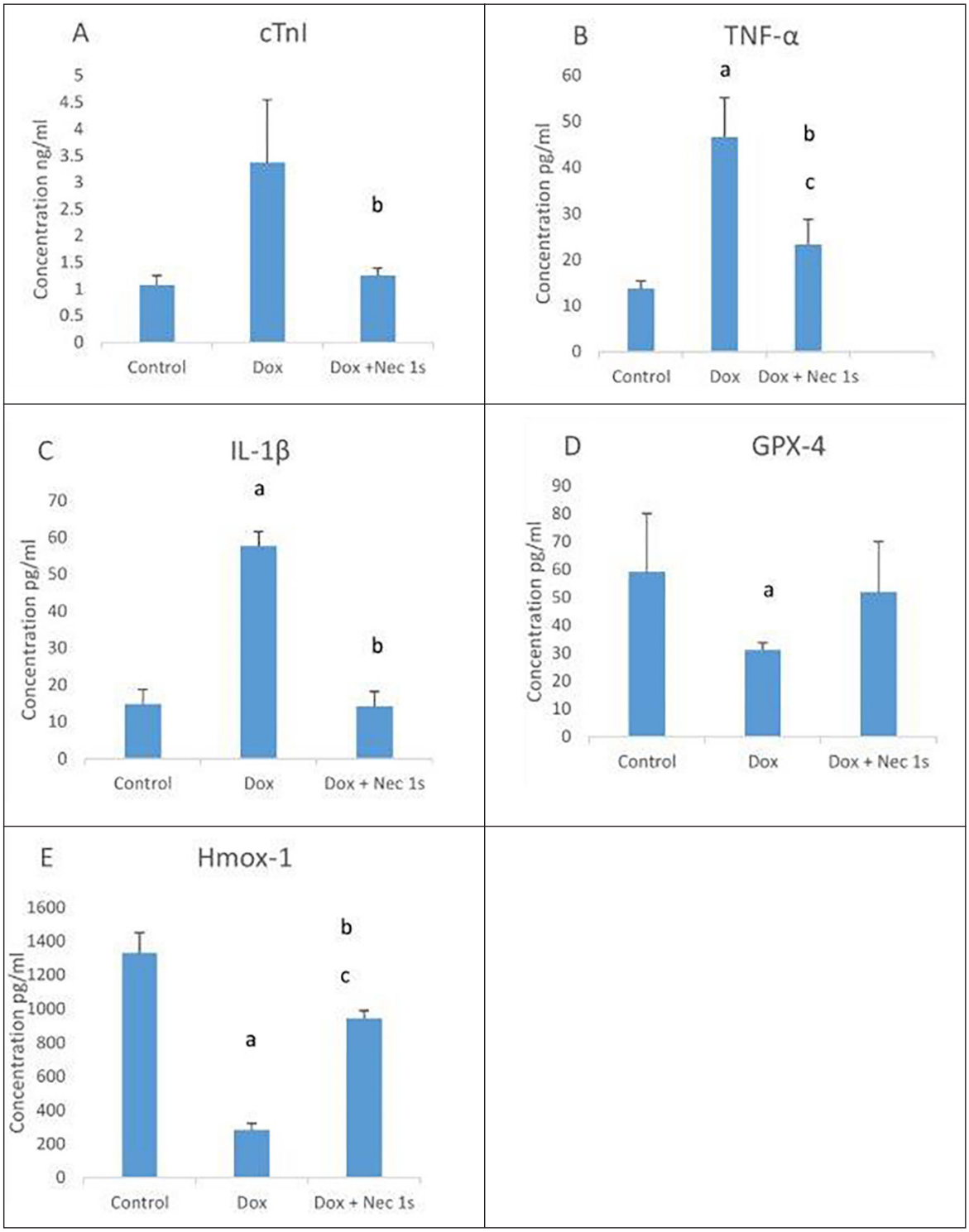

Fig. 1. Effects of Nec 1s on cardiotoxicity, inflammatory and antioxidant parameters on Dox-induced cardiotoxicity in male mice. Graph illustrates changes in (A) serum cardiac troponin I (cTnI), (B) cardiac tissue tumor necrosis factor alpha (TNF-α), (C) cardiac tissue interleukin 1-beta (IL-1β) (D) cardiac tissue glutathione peroxidase 4(GPX-4), (E) cardiac tissue heme oxygenase 1 (Hmox-1) among experimental groups. All data are presented as mean ± SD. (a) $P \leq 0.05$ for Dox vs. control; (b) $P \leq 0.05$ for Nec 1s plus Dox vs. Dox and (c) $P \leq 0.05$ for Nec 1s plus Dox vs. control

improving post-ischemic cardiac performance [16]. In the present study, Dox caused a significant decrease in Hmox1 concentration, which was consistent with the findings of Gu et al. [17], who reported that Dox-induced cardiomyocytes damage was accompanied by Hmox1 inhibition; however, a study by Qin et al. [18] showed the opposite, where Hmox1 levels were significantly increased after Dox treatment. Our study found that pretreatment with Nec-1s resulted in a considerable rise in the cardiac Hmox-1 level. The function of Hmox1

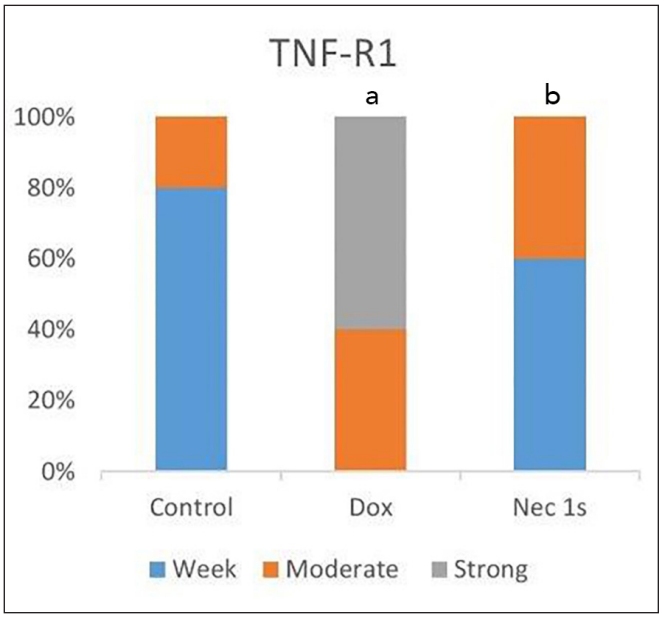

Fig. 2. Immunohistochemistry intensity score for TNF-R1, analyzed by Kruskal Wallis test. (a) $P \leq 0.05$ for Dox vs. control; (b) $P \leq 0.05$ for Nec 1s plus Dox vs. Dox

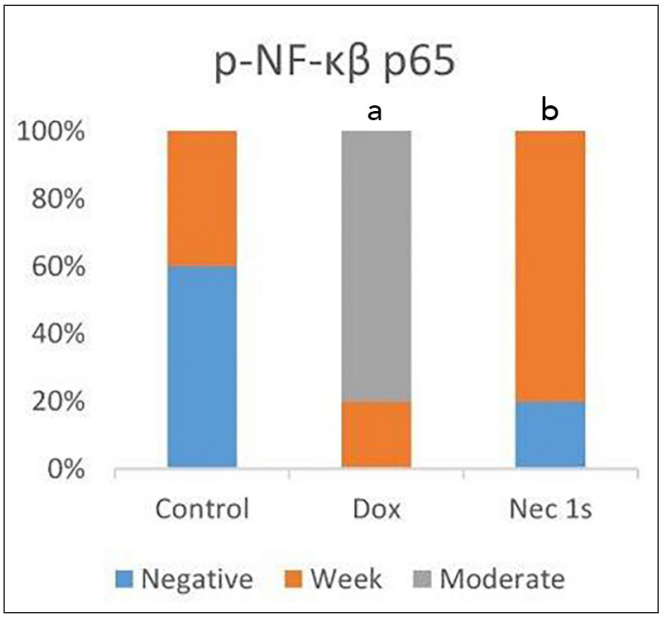

Fig. 3. Immunohistochemistry intensity score for phospho-NF-κβ p65, analyzed by Kruskal Wallis test. (a) $P \leq 0.05$ for Dox vs. control; (b) $P \leq 0.05$ for Nec 1s plus Dox vs. Dox

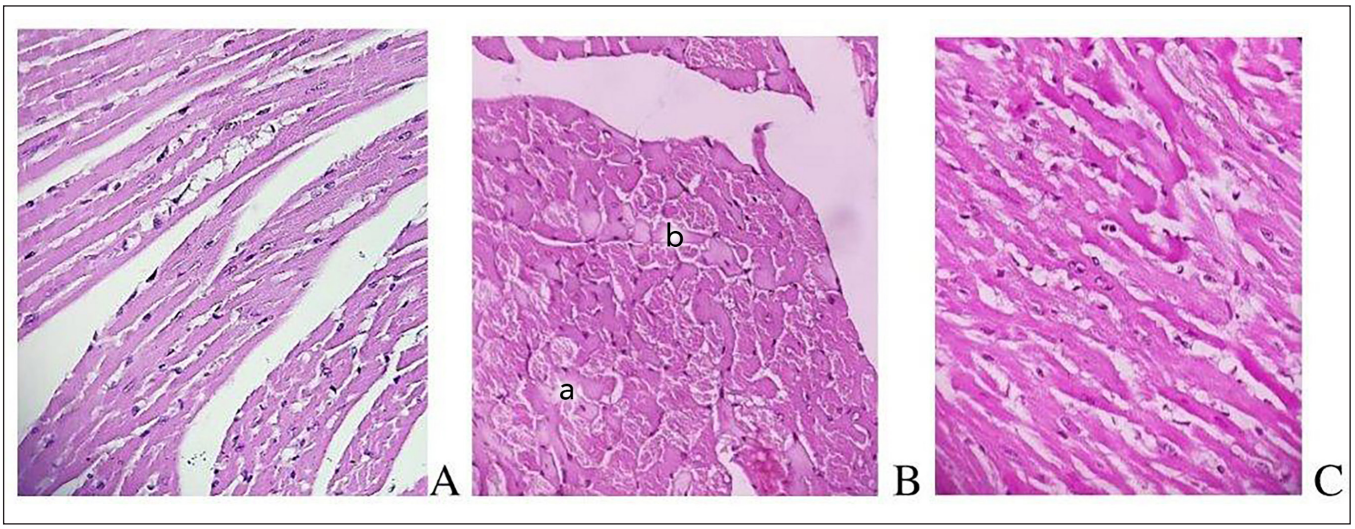

Fig. 4. Photomicrographs of myocardium sections taken at 400X magnification (A–C): (A) Control group which shows normal architecture of myocardium, (B) Doxorubicin (Dox) group which demonstrates severe lesion with myocardial congestion, myofibrillar necrosis (a) and vacuolization of the cytoplasm (b). (C) Dox plus Nec 1s group which show mild lesion with no significant vacuolization or myofibrillar necrosis.

has lately been re-examined, and both protective and detrimental functions have been identified [19-21]. According to these investigations, Hmox1 converts heme to biliverdin/bilirubin, carbon monoxide, and Fe++ [22]. Hence, it has been postulated that Hmox-1 has cytoprotective antioxidant activities against various stress-related conditions through its metabolites biliverdin/bilirubin, which may prevent lipid and protein peroxidation by scavenging ROS [23]. The second heme metabolite is carbon monoxide, which has vasodilatory, anti-inflammatory, and anti-proliferative properties in several cell types [22-24]. It may also work with NF-κβ to alter the production of anti-apoptotic proteins [22]. Fe++ is an additional metabolite of Hmox-1. Since it may interact with cell components and create free radicals, it is hazardous. This explains the pro-oxidative action of Hmox1 [19]. In the current work, Hmox1 seems to have a cardio-protective function, which is consistent with the findings of Hull et al. [25] who revealed that Hmox1 mediates cardiac protection in part via modulating mitochondrial autophagy. Regarding GPX-4, the results of the current investigation show that Dox led to a considerable downregulation of GPX-4 in cardiac tissue. These findings are consistent with those of Ta-

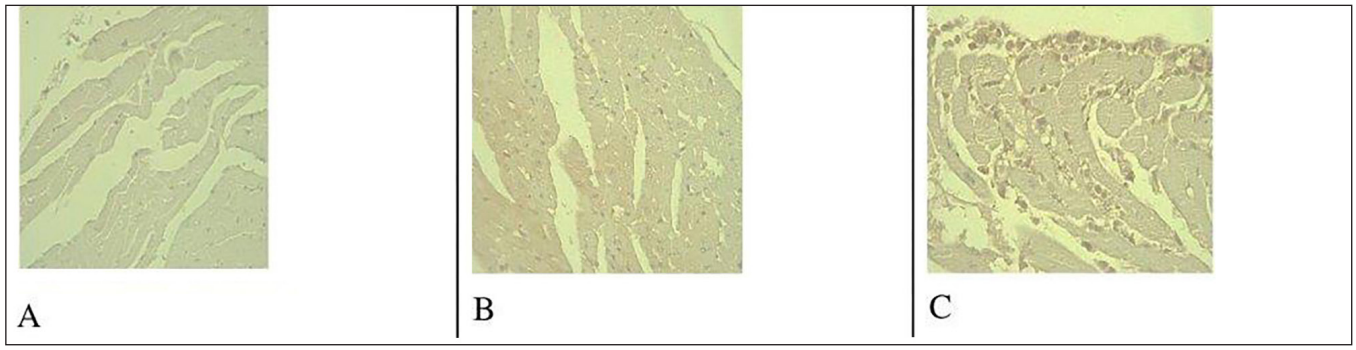

Fig. 5. Immunohistochemistry staining for TNF-R1 expression in cardiac cells (400X). A: Weak intensity stain (brown), represent control, B: Moderate intensity stain, mainly of Nec 1s pretreated group, C: Strong intensity stain, mainly noticed with Dox group

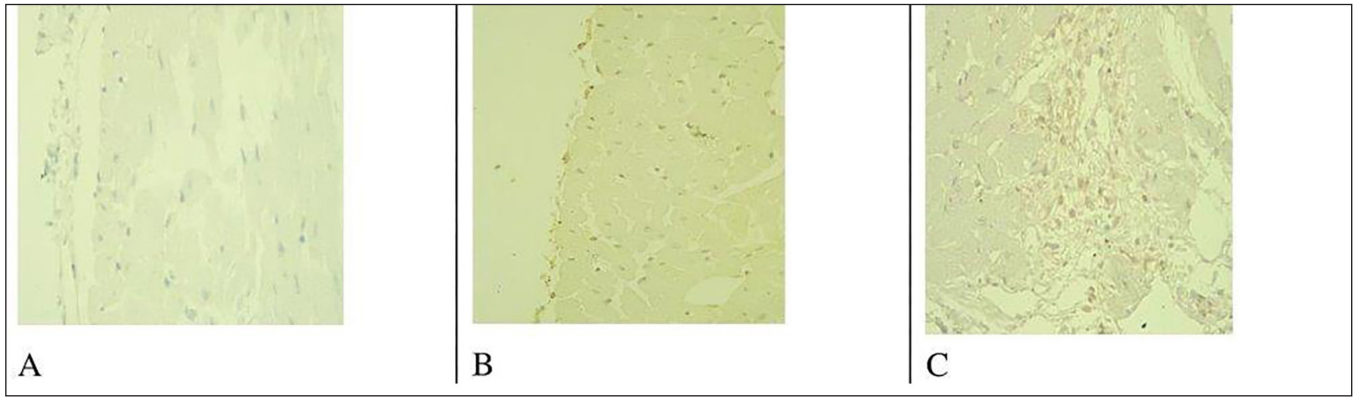

Fig. 6. Immunohistochemistry staining for phospho-NF-κβ p65 expression in cardiac cells (400X). A: Negative stain, B: very weak intensity stain (brown color), mainly noticed with Nec 1s pretreated group, C: Moderate intensity stain, mainly noticed with Dox group

dokoro et al. [26]. Another study by Li and his colleagues revealed that Dox administration was associated with downregulation of GPX-4 and Hmox1, and restoration of these enzymes was associated with cardio-protection [27]. Although pretreatment with Nec-1s caused elevation in GPX-4 level, however, this effect did not reach the level of significance. In this respect, investigations have shown that Dox exposure increases oxidative stress, hence upregulating the expression of the NF-κβ gene. As a result, more pro-inflammatory cytokines, such as TNF-α and IL-1β, are released into myocardial cells [28]. NF-κB comprises five distinct subunits, namely p-65, p-50, c-Rel, RelB, and p52. The p65:p50 represent the most abundant heterodimer that activated in response to pathological stimuli, including ligands binding to TNF-R1 and certain chemotherapeutic agents [29]. When p65:p50 is phosphorylated, it then translocate to the nucleus and starts the process of gene transcription [30]. TNF-α is an inflammatory cytokine that acts in complex with its receptors, TNF-R1. The TNF/TNF-R1 complex represents a crucial element of the inflammatory response to Dox. [31], this was confirmed by our current study, where Dox caused a significant increase in cardiac expression of p-NF-κβ p65 and TNF-R1 (as evidenced by increasing intensity of immunostaining), together with increase in cardiac levels of TNF-α and IL-1β. These effects were modified partly or completely by pretreatment with Nec-1s. Acute Dox injection caused extensive cytoplasmic vacuolization, congestion of cardiac fibers, and myofibrillar necrosis. Many prior studies have shown similar cellular and structural changes [32]. The histopathological results of our research indicated that Nec-1s has the potential to attenuate cardiac tissue damages brought on by Dox. This was proven by the considerable drop in severity score when compared to the Dox group.

CONCLUSIONS

Our findings demonstrate that pretreatment with Nec 1s, a potent necroptosis inhibitor, protected against acute Dox-induced cardiotoxicity. This cardioprotective effect was reflected by reduction of cTnI level, amelioration of inflammation by inhibition of expression of cardiac phospho-NF-κβ p65 and TNF-R1, reduction of TNF-α and IL-1β levels with partial restoration of antioxidant enzymes, GPX-4 and Hmox1. These alterations were consistent with histopathological features that showed preservation of normal myocardial morphology.

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ORIGINAL ARTICLE

INFLUENCE OF CHOLESTEROL ENRICHED DIET ON GENES EXPRESSION ENCODING BONE MORPHOGENETIC PROTEIN-2 AND OSTEOCALCIN IN MOUSE MANDIBLE

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ABSTRACT

The aim: To evaluate the mRNA expression of the key regulators of osteogenesis – osteocalcin and BMP-2 in the mouse embryos mandible (17th day of pregnancy) which were borne by females on high-cholesterol diet for 30 days before fertilization and throughout pregnancy.

Materials and methods: Experimental hypercholesterolemia (2%) was simulated by adding Cholesterol to the diet for 60 days. In experiment were used 40 mature female white mice that were randomly divided to control and experimental groups. The control group were fed with standard chow diet, the experimental group with diet with cholesterol enriched diet (with addition of 2 grams of Cholesterol per 100 grams of standard chow). The mandibles of mouse embryos (E–17) were examined by using molecular genetic methods.

Results: In control group the relative level of BMP-2 mRNA / actin mRNA was 27.0±2.82, the relative level of and osteocalcin mRNA / actin mRNA was 30.5±6,28. In the jaws of animals in the experimental group with cholesterol enriched diet, the expression relative level of BMP-2 was 30.9±5.81 that is by 14,4% higher than in control group. Therefore, the expression level of osteocalcin, on the contrary, decreased by 22.3% and was 23.7+5.31.

Conclusions: Our study report influence of the cholesterol enriched diet (2%) on mRNA expression of BMP-2 and osteocalcin encoding genes. The embryos from mouse on cholesterol enriched diet (2%) had increased level of BMP-2 gene expression, however significantly decreased level of osteocalcin gene expression.

KEY WORDS: bone morphogenetic protein-2, osteocalcin, mouse embryos mandible, hypercholesterolemic diet, cholesterol

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INTRODUCTION

Adequate and balanced nutrition of the pregnant woman ensures the normal fetal development and is the basis for the formation of organs and tissues of the oral cavity in the baby. Nutrition during pregnancy play an important role in tooth development, that basically include influence on primary tooth formation and the start of mineralization. More over in future may account for dental caries susceptibility in children [1].

In all trimesters, more than a half of the sample group reported fat intakes as percentages of energy that exceeded the acceptable range of 20–35% [2]. These results were similar to those [3] in which a third of the 1533 studied pregnant women had total fat intakes as a percentage of energy exceeded the Acceptable Micronutrient Distribution Range. Furthermore, a meta-analysis [4] of the studies also found that the average fat intakes in pregnant women also above recommendations (as percentages of energy intake) and were 35.0% to 37.1%, in accordance with the results [2].

Unbalanced and poor nutrition of pregnant women increases the risk of dental caries in the unborn child by forming low caries susceptibility of the embryo tooth tissues, fetal immaturity and enamel hypoplasia of the baby, and as a result, leading to caries of deciduous teeth in children [5]. However, still is not enough studies about particular effects of maternal diet on dental health of child in order to make evidence-based statements. There are studies that show that High fat diet (HFD) has deleterious effect on bone micro-architecture [6 – 7].

A constant component of fatty foods is cholesterol. Cholesterol occurs naturally only in foods of animal origin. The highest concentrations are found in liver and egg yolk, but red meats, poultry (especially the skin), full-fat milk, and cheese make significant contributions to the diet [8]. Dietary cholesterol was implicated in increasing blood cholesterol levels [9].

Bone morphogenetic protein and osteocalcin play a crucial role in stages of odontogenesis and tooth germ

early mineralization. Many studies investigated that BMP-2 is needed to start the differentiation of stem cells from exfoliated deciduous teeth into odontoblasts [10-12]. Some studies show that one of the growth factors for the isolated dental papilla and odontoblasts in future was BMP-2 [13]. Also, BMP-2 has been found to induce differentiation of the cells of follicle into a cementoblasts or osteoblasts phenotype [14]. One more of stimulating effect of BMP-2 is proliferation and then differentiation of pulp cells into odontoblast cells [15], consequently, BMP-2 are able to induce osteodentine and tubular dentine, in turn, growth hormone is able to induce BMP-2 in dental pulp fibroblasts [16].

During tooth development, BMP-2 is initially expressed in the oral epithelial cells at embryonic day 13 (E13), and at later stages of tooth development its expression shifts to the mesenchymal dental papilla and becomes more intense with dentinogenesis [17].

Bone gamma-carboxyglutamate protein (BGLAP, osteocalcin) is protein that is secreted by mature osteoblasts; thus, expression of BGLAP is an indicator for bone function. One of study paid attention to a vitamin K-dependent matrix protein – osteocalcin that is produced by dentine odontoblasts and in bone tissue and has ability to binding to hydroxyapatite. Finding form this study suggest key role of osteocalcin in dentino- and cementogenesis, bone and periodontal ligament remodeling as well as mineralization [18].

Researches said that osteocalcin was expressed only during the later stages – at the maturation stage of enamel formation, and was detected in odontoblasts and their processes within the extracellular matrix. Our data show that osteocalcin and osteonectin, coexpressed in bone and teeth, may diverge in their functional involvement in relation to the binding specificities of the cellular and extracellular components of dental mineralized tissues [19].

There some studies have linked imbalanced maternal nutrition with violated fetal odontogenesis, but it unknown whether excessive cholesterol intake during pregnancy alter teeth germination disorders via influence on expression of two important proteins of tooth development and mineralization, BMP-2 and osteocalcin, that are encoding by BMP-2 and Bglap genes, correspondingly.

THE AIM

The aim of this study was to evaluate the mRNA expression of the key regulators of osteogenesis – osteocalcin and bone morphogenetic protein 2 (BMP-2) in the mouse embryos mandible (17th day of pregnancy) which were borne by females on high-cholesterol diet for 30 days before fertilization and throughout pregnancy.

MATERIALS AND METHODS

For decades, the laboratory mouse has been the preferred model organism for the study of human biology and diseases. Humans and mice share a very similar genetic background, and around 90% of both genomes can be partitioned into regions of conserved synteny. The extrapolation of the obtained results to humans is theoretically possible due to the great described genes sequence similarity between humans and mice [20].

Forty mature female white mice were housed in cages with controlled conditions and free access to water (20–24°C, 50–60% relative humidity, artificial 12-h light – 12-h dark cycle). Mice were randomly divided to control and experimental groups (20 mice in each group). The control group were fed with standard chow diet, the experimental group with diet with cholesterol enriched diet (with addition of 2 grams of Cholesterol per 100 grams of standard chow).

Adult females after 30 days of experiment in proestrus or estrus cycle phase used in this study were paired overnight with vigorous sexually experienced adult males in proportion 4 to 1. Successful mating was confirmed by the presence of sperm in the vaginal smear the following morning (07:00–08:00) and this day was considered day 1 of pregnancy. Only sperm positive females were used in the study. The pregnant mice were assigned into various groups according to the design of the experiment. Throughout pregnancy females were kept in cages and fed with standard chow for control group or cholesterol-enriched chow for experimental group.

Period of mice embryotic development between E16.5 and E18.5 is known as bell stage. During this stage influence of BMP-2 and Bglap genes is crucial for odontogenesis and mineralization [21]. That is why 17th day of embryotic development was chosen as a day of the end of experiment in order to achieve the aim of this study. 6 pregnant mice from each group were exposed by carbon dioxide on the 17th day of pregnancy (E17). Mandibles of 17 embryonic days old mouse (E17) were excised to provide molecular-genetic investigation. All procedures with animals were in accordance with the «Rules and Regulations for Carrying Out Animal Research Work».

Mandibles of 17 embryonic days old mouse (E17) were used for phenol-chloroform extractions of RNA samples. Sigma-Aldrich (USA) reagents were used for these purposes. Concentration of RNA in samples was analyzed using the Thermo Scientific NanoDrop™

1000 Spectrophotometer (USA) and Thermo Scientific RevertAid First Strand cDNA Synthesis. 200-300 μg of total RNA were used for reverse transcription using First Strand cDNA Synthesis Kit (Fermentas, Lithuania) and $(Oligo(dT)_{18}$ Primer the oligo(dT)18. PCR amplification of cDNA from RNA was performed.

BMP-2 and Bglap expression was measured and performed by real-time PCR. Next primers were used:

BMP-2 Up: 5`-GTGGAGGAACTTCCAGAGATGA-3`,

BMP-2 Dw: 5`-CTGCAGATGTGAGAAACTCGTC-3`,

Osteocalcin Up: 5`-CAGGAGGGCAATAAGGTAGTGA-3`,

Osteocalcin Dw: 5`-CAGGGTTAAGCTCACACTGCTC-3`.

For quantitative analysis, real-time PCR was conducted with a using SYBR Green PCR Master Mix (Thermo Fisher Scientific) – 20 μL with 25 pM of each primer. The thermal cycling conditions (with initial denaturation and AmpliTaq Gold® DNA polymerase activation) were as follows: 10 min at 95°C followed by 50 cycles of 95°C for 15 s, 60°C for 60 s (for osteocalcin 61°C).

In order to distinguish among PCR product melting curve analysis was used. Melting stage consisted of gradual temperature increase from 60 or 61°C to 94°C. By the temperature increase, DNA molecules dissociate and fluorescence of DNA-SYBR Green complex starts to reduce.

7500 Fast Real-time PCR Software by Applied Biosystems was used to perform the data of this investigation.

Statistical analyses. Data were analyzed with Origin 7.0 and Excel 2000. Shapiro-Wilk test was used for checking the data for normality. The study of difference was caried out by Mann-Whitney two-tailed test. Differences were considered significant at $P < 0.05$.

RESULTS

To determine the influence of hypercholesterolemia on BMP-2 and osteocalcin quantity we examined of mRNA expression of encoding genes of these proteins, as a key factor of odontogenesis.

Amplification products (actin, BMP-2 and osteocalcin) concentration relate to curve of amplification cycle number and curve of dissociation amplification products are shown in fig. 1.

The real-time PCR analysis showed that in control group with standard chow the expression of BMP-2 and osteocalcin genes in the mandible of mouse embryos genes was at approximately the same level. In control group the relative level of BMP-2 mRNA / actin mRNA was 27.0±2.82 (fig. 2), the relative level of and osteocalcin mRNA / actin mRNA was 30.5±6,28 (fig. 3). However, in the jaws of animals in the experimental group with cholesterol enriched diet, the expression relative level of BMP-2 tended to increase and was 30.9±5.81 ($p=0.53$; $p>0,05$) that is by 14,4% higher than in control group (fig. 2). Therefore, the expression level of osteocalcin, on the contrary, decreased by 22.3% and was 23.7+5.31 (fig. 3).

The analysis of the obtained data allows us to assume that the hypercholesterol diet to some extent changes the expression of BMP-2 and osteocalcin genes, and these changes are in different directions. According to the function of BMP-2 as a key factor in the differentiation of odontoblasts, it can be assumed that increasing its expression will accelerate odontogenesis. A decrease in the expression of the osteocalcin gene, which ensures mineralization in the tissues of the tooth germ, can lead to insufficient hydroxyapatite saturation of the hard tissues of the forming tooth, and, as a result, the eruption of teeth with a reduced level of mineralization.

DISCUSSION

There is a lot of data on the effect of hypercholesterolemia and of statins on the expression of BMP-2 and osteocalcin. The most indicative data were obtained indirectly through the study of the statins effect (known as hypercholesterolemic agents) on the processes of calcification and ossification. One of the epidemiological studies have shown that statins increase bone mineral density and reduce the risk of bone fractures [22]. Statins can directly stimulate BMP-2 expression and osteoblast differentiation, while inhibiting osteoclast activity and osteoblast apoptosis [23].

There are some studies that show that concentration of osteocalcin in the blood serum depends on the concentration of cholesterol. People with hypercholesterolemia and high blood sugar (metabolic syndrome) tend to have lower osteocalcin level [24]. Also, hypercholesterolemic drugs (rosuvastatin) increase serum osteocalcin levels, although this effect does not depend on lowering cholesterol levels [25].

Unfortunately, these data do not provide a direct answer how exactly hypercholesterolemia influence on tooth development, but lowering cholesterol levels under the influence of statins may suggest that elevated cholesterol levels may adversely affect BMP-2 expression and bone mineral density in general. D.L. Franklin [26] showed that odontoblasts in the early stages of development contain much less cholesterol than fully differentiated cells. Cholesterol content and distribution significantly affect the fluidity of the odontoblast cell membrane, and the low cholesterol content in low-differentiated odontoblasts provides budding of the matrix vesicles required for tooth formation. Earlier, similar data were obtained: the cholesterol content is much higher in permanent teeth compared to the teeth that are formed [27].

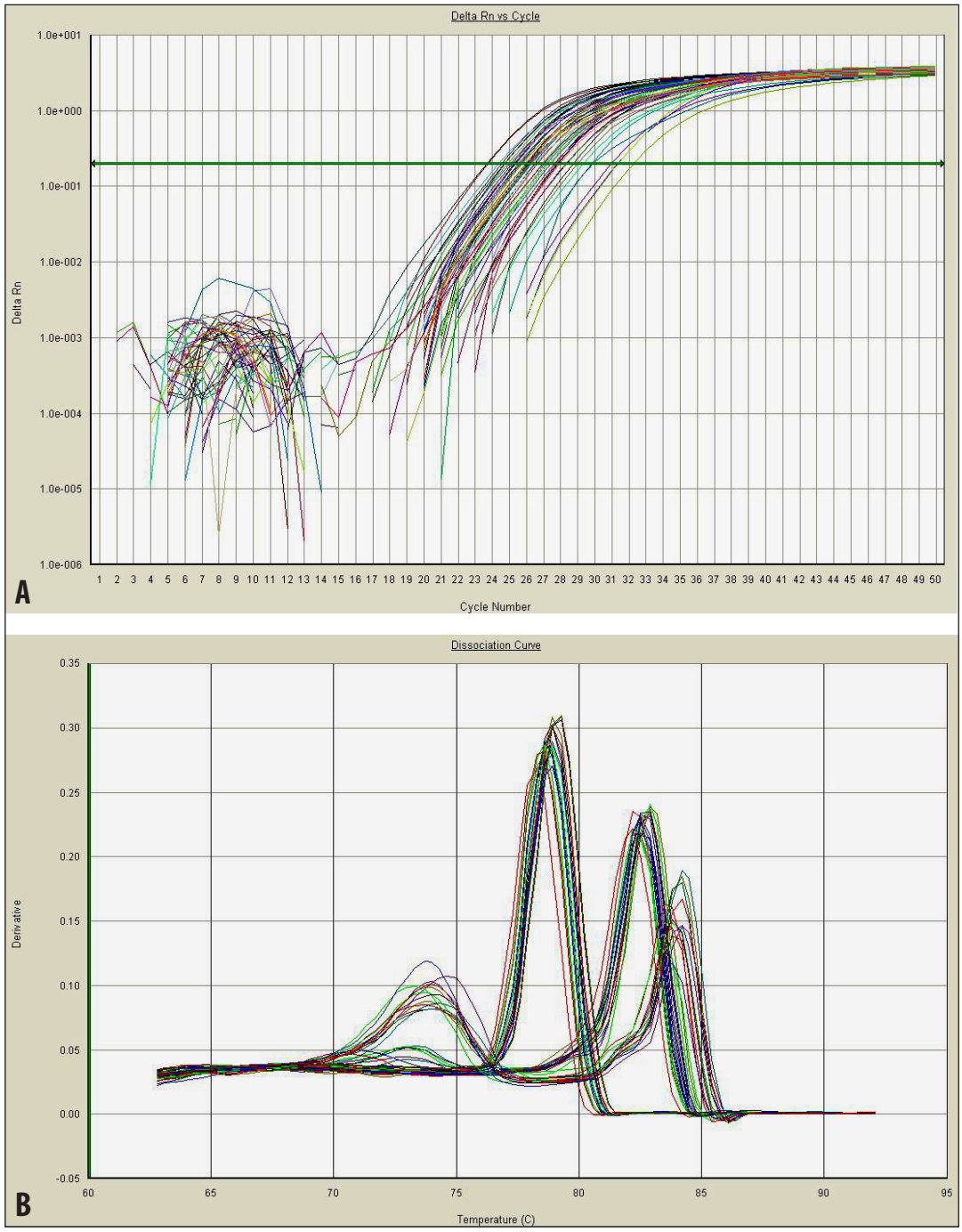

Fig. 1. A: Concentration of amplification products – actin, BMP-2 and osteocalcin correspondingly with curve of amplification cycle number; B: Concentration of amplification products – actin, BMP-2 and osteocalcin correspondingly with curve of dissociation amplification products.

Analysis of the obtained data suggests that the hypercholesterolemic diet alters, to some extent, the expression of BMP-2 and osteocalcin genes, and these changes are multidirectional. Based on the function of BMP-2, as a key factor in the differentiation of odontoblasts and function of osteocalcin as inducer of dentine and enamel mineralization, we can assume that increase of its expression will accelerate odontogenesis. In this case, the decrease in the expression of the osteocalcin gene, which provides mineralization in tooth tissues, can lead to insufficient saturation with hydroxyapatite of hard tissues of the tooth, which is formed, resulting in the eruption of teeth with low mineralization.

The date from some studies showed that lower levels of osteocalcin are not sufficient to markedly influence dentinogenesis but enamel mineralization is more sensitive to osteocalcin presence. This difference could manifest because of different character of mineralization stages of this two tooth hard tissues – enamel crys-

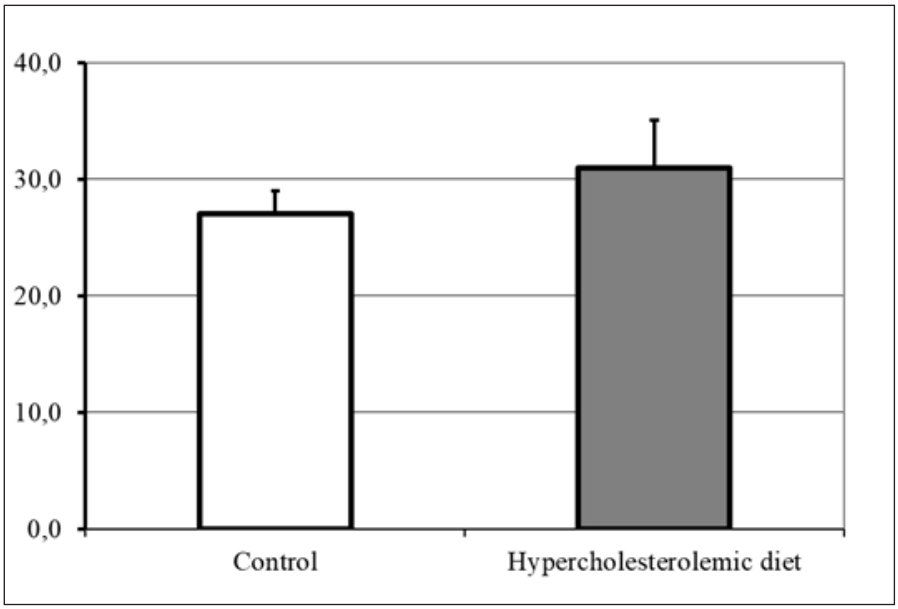

Fig. 2. mRNA expression of BMP-2 relative to actin in mandible of mouse embryos in control with standard chow and experimental cholesterol enriched diet groups. Research groups are represented horizontally, the relative level of BMP-2 mRNA / actin mRNA in are represented vertically

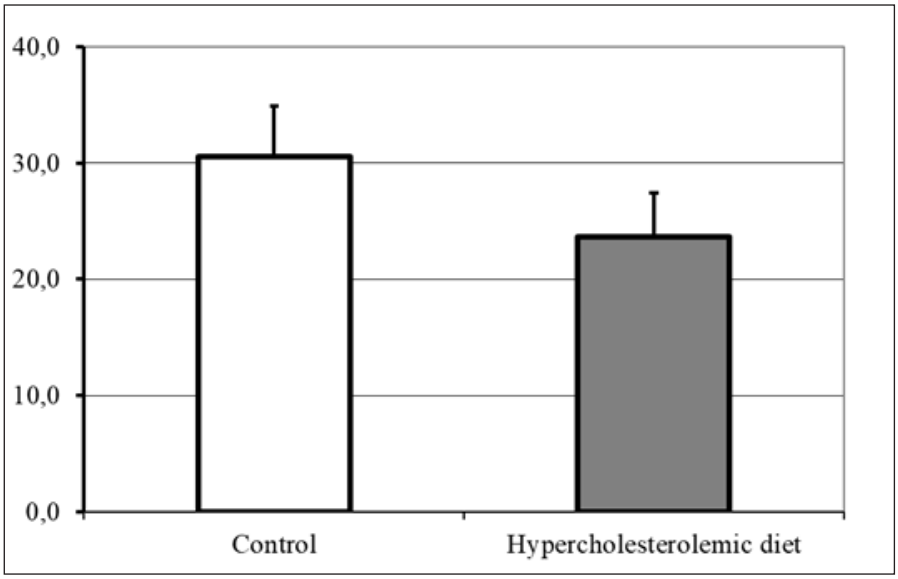

Fig. 3. mRNA expression of osteocalcin relative to actin in mandible of mouse embryos in control and experimental cholesterol enriched diet groups. Research groups are represented horizontally, the relative level of osteocalcin mRNA / actin mRNA in are represented vertically

tals in secretory stage of enamel formation are growing in a non-collagenous enamel matrix contained within a tightly controlled enamel compartment, formed by the mineralized dentin and the layer of secretory ameloblast [28].

In this study we have received evidence that in mandibles of mouse embryos from mice that had high-cholesterol diet for 30 days before fertilization and throughout pregnancy had tendency to increasing BMP-2 expression and decreasing osteocalcin gene expression. Decreased level of osteocalcin gene expression could leads to failure in tooth germ early mineralization.

Results of our study may suggest that maternal hypercholesterolemia has the specific influence on health of the oral cavity of future child through altering saturation of hydroxyapatite and normal process of tooth mineralization during tooth formation. In turn mineralization defects could result in amelogenesis imperfecta, low level of enamel mineralization and early caries after tooth eruption.

CONCLUSIONS

Our study report influence of the cholesterol enriched diet (2%) on mRNA expression of BMP-2 and osteocalcin encoding genes. The embryos from mouse on cholesterol enriched diet (2%) had increased level of BMP-2 gene expression, however significantly decreased level of osteocalcin gene expression. The histopathological changes investigation in tooth germs in mandible of 17-days mouse embryos allows comparison of genetic and pathomorphological changes and helps to determine the functional significance of changes in expression of the studied genes.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

SCREENING OF MEDICINAL AND AROMATIC PLANTS EXTRACTS FOR THE SYNERGISM WITH FLUCONAZOLE AGAINST *CANDIDA ALBICANS* AND *CANDIDA TROPICALIS* FUNGI ASSOCIATED WITH DENTURE STOMATITIS

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ABSTRACT

The aim: To conduct a primary screening of the ability of aqueous-ethanol extracts of medicinal plants to enhance the effect of fluconazole against resistant strains of *Candida sp.* associated with denture stomatitis, to justify the potential use of combined antifungal therapy.

Materials and methods: 40 biochemical tests using the VITEK 2 system with the use of VITEK 2 YST ID card (Biomerieux, France). The computer programs UTHSCSA ImageTool 2.0 and Microsoft Office Excel 2003 were used for statistical processing of the results.

Results: 114 extracts out of 166 studied ones (68.7 ± 0.28%) showed direct antifungal activity in relation to *C. tropicalis strain*, 74 extracts (44.6 ± 0.30%) turned out to be highly active (d IZ > 10 mm). Only 50 extracts out of 166 studied ones (30.1 ± 0.28%) showed antifungal activity against *C. albicans* strain, 26 extracts (15.7 ± 0.22%) were highly active (d IZ > 10 mm). Significant direct antifungal activity both against *C. albicans* strain and *C. tropicalis strain* was demonstrated by the extracts of the leaves of *Sophora japonica*, thallus of Mnium cuspidatum Hedw. (*M.silvaticum Lindb*.), herbs of *Euphorbia amygdaloides L*., *Lathyrus niger (L.) Bernh*., *Betonica officinalis L*. s. l., flowers of *Primula officinalis Hill*., roots of *Scrophularia nodosa L*.

Conclusions: 1. Aqueous-ethanolic extracts of medicinal and aromatic plants of Ukrainian flora have direct antifungal activity against azole resistant *C. albicans* and *C. tropicalis* (44,6±0,30% and 15,7±0,22% of tested extracts respectively) associated with denture stomatitis as well restore their sensitivity to fluconazole (44,6±0,30% and 15,7±0,22% of extracts respectively).

KEY WORDS: plant extracts, Fluconazole, Candida fungi, resistance to antifungal agents, synergism of antifungal action

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INTRODUCTION

Candida albicans is one of the most common and virulent representatives of *Candida* fungi which causes fungal lesions of the mucous membranes predominantly in people with compromised immune system. *Candida* resistance to classical antifungals such as polyenes, azoles, allylamines is a serious practical problem. Imidazole and triazole are the most commonly used antifungal agents for candidiasis today. Their mechanism of action lies in the inhibition of ergosterol biosynthesis which is indispensable for maintaining the structural integrity of fungal cell membranes. Activation of elimination systems is often associated with changes in the structure of fungal membranes, leading to a decrease in the supply of azoles into the fungal cell [1,2]. Another innovative approach is also noteworthy. It consists in an increase in *Candida* sensitivity to classical antifungals by neutralizing their resistance determinants. An example of such a strategic approach is the combination of penicillins, cephalosporins and carbapenems with β-lactamase inhibitors in case of bacterial infections. Recently, a number of synthetic and natural substances capable of enhancing the Fluconazole effect on resistant *Candida* strains have been described [3-5].

THE AIM

To conduct a primary screening of the ability of aqueous-ethanol extracts of medicinal plants to enhance the effect of fluconazole against resistant strains of *Candida sp.* associated with denture stomatitis, to justify the potential use of combined antifungal therapy.

MATERIALS AND METHODS

166 aqueous-ethanol extracts from different parts, namely, flowers, leaves, aerial parts, roots, fruits and inflorescences of medicinal and aromatic plants were studied. The dried crushed raw material was extracted by maceration with 90% aqueous ethanol in accordance with the require-

ments of the State Pharmacopoeia of Ukraine at room temperature for 2 weeks (raw material / extraction solvent ratio 1:10). The residue of the raw material was filtered off and the volume was adjusted to the initial level with pure extraction solvent in order to obtain the final extracts. Testing was performed on clinical strains of *C. albicans* and *C. tropicalis* fungi isolated from the surface of the denture bed of patients with denture stomatitis. The cultures were identified according to a set of morphological and cultural properties and on the basis of 40 biochemical tests using the VITEK 2 system with the use of VITEK 2 YST ID card (biomerieux, France). The cultures sensitivity to classical antifungals was determined by the disk-diffusion method, and the sensitivity to fluconazole was determined by the micromethod of serial dilutions in YPD liquid medium with interpretation of results according to EUCAST (European Committee on Antimicrobial Susceptibility Testing), Version 10.0, 2020-02-04 [6] in addition to the disk-diffusion method. The minimal fungistatic concentrations (MFC) of Fluconazole were determined on the basis of the analysis of culture growth curves obtained by hourly recording of changes in optical density of the medium using a multimode spectrophotometer for microplates Synergy ™ HTX S1LFTA (BioTek Instruments, Inc., USA).

Screening of extracts antifungal activity was performed using agar diffusion micromethod [6]. The inhibition zones (IZ) diameters less than 7.00 mm were not taken into account considering the results of control experiments in order to exclude the effect of extraction solvent (90% ethanol) on the growth of test cultures.

Similar inoculations on Sabouraud agar were performed with the addition of antifungal agent at final concentrations of $^1/_4$, $^1/_8$ or $^1/_{32}$ MFC for each test strain for extracts screening for the synergism of antifungal action with Fluconazole. The diameters of cultures IZ under the influence of plant extracts on medium without antifungal agent and on media with subfungistatic concentrations of Fluconazole were determined after 48 hours of incubation at the temperature of 37° C.

The computer programs UTHSCSA ImageTool 2.0 and Microsoft Office Excel 2003 were used for statistical processing of the results. The reliability of the increase in the inhibition zones of fungi in the presence of combinations of different concentrations of fluconazole with plant extracts was evaluated using the Student's t-test and one-way analysis of variance (ANOVA).

RESULTS

The presented research was performed on modern clinical strains of *Candida* isolated from dental patients with complications after denture treatment. The clinical strains of *Candida* selected for screening were identified as *C. albicans* and *C. tropicalis* by the VITEK 2 system. The resistance profiles of both strains to antifungal agents of different groups were established by the disk-diffusion method (Table I). Weak fungistatic effect was observed around disks with Imidazole and Triazole (including Fluconazole). However, the disk-diffusion method is considered low-informative for the detection of antifungal resistance and its results are difficult to be clinically interpreted from the perspective of the choice of therapeutic drug and the required dosage regimen [9]. Therefore, there are no generally accepted criteria for interpreting its results. Nevertheless, the obtained evidence showed the polyresistance of both strains to classical antifungals being a serious practical problem in clinical practice.

Method of serial dilution and E-test [9] are considered more reliable for determination of antifungal resistance. Therefore, both test strains resistance to Fluconazole was confirmed by microplating method in YPD liquid medium. Fluconazole MFC was determined in the presence of well known azoles efflux inhibitors Haloperidol [7] and Chloropromazin, in order to determine the resistance mechanism (Table II). According to the criteria of EUCAST (European Committee on Antimicrobial Susceptibility Testing), Version 10.0, 2020-02-04

Table I. Comparative Characteristics of the Sensitivity of the Used Fungi Clinical Strains to Antifungal Agents (IZ Diameters, mm)

Antifungals	*C. albicans*		*C. tropicalis*		Breakpoint (dose-related sensitivity)
	Fungicidal activity	Fungistasis	Fungicidal activity	Fungistasis	
Amphotericin B	6	9	6	6	10-14
Nystatin	14	14	14	14	17-24
Clotrimazole	6	23	6	15	12-19
Ketoconazole	6	25	6	20	15-18*
Fluconazole	6	30	6	24	15-18
Itraconazole	6	15	6	11	15-18*
Terbinafine	6	6	6	10	12-19

Note: * – precarious indicater

Table II. Comparative Characteristics of Sensitivity of the Used Clinical Strains of *Candida Fungi* to Fluconazole

	C. albicans		*C. tropicalis*	
	MFC, μg/ml	**Decrease multiplicity of FCZ MFC**	**MFC, μg/ml**	**Decrease multiplicity of FCZ MFC**
Fluconazole (FCZ)	64	-	32	-
Chloropromazin	125	-	250	-
Haloperidol	500	-	500	-
FCZ + Chloropromazin ($^1/_8$ MIC)	32	2	4	8
FCZ + Haloperidol ($^1/_8$ MIC)	8	8	2	16

Table III. Antifungal Activity of Plant Extracts in the Presence of Subfungistatic Concentrations of Fluconazole in Relation to *C. Albicans* and *C. Tropicalis* Strains with Different Mechanisms of Resistance (Diameters of IZ, mm)

Extracts	**Plant part**	***C. albicans* (FCZ MFC 62.5 μg/ml)**				***C. tropicalis***			
		control (without FCZ)	**FCZ ($^1/_4$ MFC)**	**FCZ ($^1/_8$ MFC)**	**FCZ ($^1/_{32}$ MFC)**	**control (without FCZ)**	**FCZ ($^1/_4$ MFC, 125 μg/ml)**	**FCZ ($^1/_8$ MFC, 125 μg/ml)**	**FCZ ($^1/_{32}$ MFC, 125 μg/ml)**
Control (90% ethanol)		4.54±0.57	6.12±0.44	6.64±0.63	6.40±0.96	4.59±0.28	7.53±1.60	6.86±0.68	5.29±1.29
Calendula officinalis L.	inflorescence	5.40±0.71	5.63±0.56	10.55±1.40**	10.43±0.79**	5.81±0.90	17.19±4.59**	10.75±1.04**	11.79±2.87**
Rudbeckia laciniata L.	inflorescence	4.00±0.03	4.00±0.03	7.33±2.28	8.97±0.58*	4.94±1.06	11.55±1.97**	8.19±0.51*	10.82±3.84**
Matricaria recutita L.	inflorescence	9.29±0.31	7.72±0.78	7.39±0.65	16.50±1.54**	6.15±1.52	26.85±11.77**	6.31±0.70	22.59±6.44**
Limonium meyeri (Boiss.) O. Kuntze	stems and inflorescence	7.23±1.97	6.70±0.77	7.28±0.91	13.38±0.91**	5.60±0.37	9.48±1.45*	8.48±2.56*	6.11±1.09
Crataegus monogyna Jacq.	flowers	7.19±0.76	7.51±0.30	9.06±1.15*	10.35±1.96**	6.78±0.87	12.54±1.19**	7.49±1.02	11.57±2.58**
Potentilla repens L.	aerial part	7.25±0.71	5.67±0.99	11.58±1.48**	11.63±0.65**	5.81±0.87	11.37±2.61**	8.06±0.77*	8.32±1.57*
Agrimonia eupatoria L.	aerial part	4.00±0.03	4.00±0.03	7.45±0.67	8.55±1.20*	3.39±0.66	8.46±0.76*	10.65±2.36**	12.92±1.97**
Agrimonia eupatoria L.	roots	5.61±0.93	14.28±0.48**	11.45±1.10*	7.29±2.07	6.69±1.86	9.45±2.76*	13.30±1.45**	13.77±3.31**
Sanguisorba officinalis L.	aerial part	5.06±0.74	7.58±0.97	8.57±0.39*	8.24±2.08*	5.02±0.83	11.96±3.28**	10.67±3.41*	8.43±0.58*
Thymus serpyllum L.	aerial part	4.96±1.39	16.91±2.81**	8.07±1.08*	8.39±1.59*	5.96±0.64	11.67±1.97**	11.12±2.18**	8.20±1.75*
Eupatorium cannabinum L.	roots	7.46±0.99	9.52±0.62	5.18±0.72	4.91±0.71	4.15±0.66	18.53±3.18**	11.57±0.88**	12.31±2.87**
Melilotus albus Medik.	aerial part	5.94±0.74	11.43±0.90**	6.37±0.85	10.51±1.39**	9.04±1.84	9.13±1.59	11.21±3.36	14.75±2.24*
Peucedanum ruthenicum Bieb.	leaves	6.39±1.07	8.01±0.60*	9.04±0.82*	15.84±0.82**	5.80±0.60	14.12±0.89**	11.39±0.60**	7.61±1.97
Piper nigrum L.	fruit	4.00±0.03	12.09±1.91**	15.47±1.39**	4.10±0.96	6.82±1.02	26.40±9.00**	6.96±2.06	19.82±5.55**
Sophora japonica L.	leaves	10.07±3.40	5.26±0.57	11.25±2.40	10.42±1.92	10.81±0.57	10.61±1.67	10.17±2.51	9.83±0.94
Salix aurita L.	leaves	7.40±1.06	4.63±0.20	13.77±0.87**	10.77±0.71*	5.26±0.10	11.77±0.55**	8.10±1.54*	13.20±3.18**
Juglans regia L.	leaves	6.59±1.19	12.68±1.43**	7.04±0.99	14.55±0.78**	6.59±1.19	11.67±7.52**	8.14±1.04	10.60±1.35*
Geranium phaeum L.	aerial part	9.33±2.45	8.62±2.04	8.82±0.44	5.61±1.11	5.45±0.48	13.19±1.31**	18.61±4.45**	11.13±1.16*
Polygonatum verticillatum (L.)	roots	16.64±1.94	9.92±1.23	13.97±1.54	11.45±1.04	6.00±0.48	9.84±3.08*	11.34±1.59**	9.52±2.67*
Potentilla repens L.	roots	7.25±0.71	5.67±0.99	11.58±1.48*	11.63±0.65*	5.81±0.87	11.37±2.61*	8.06±0.77*	8.32±1.57
Polytrichum commune Hedw.	aerial part	7.43±2.31	8.06±0.55	11.11±0.83*	16.37±1.21**	9.40±0.87	7.19±1.62	10.33±1.11	6.56±0.65
Leucobryum glaucum (Hedw.) Aongstr.	aerial part	7.05±0.68	6.41±0.76	14.84±1.21**	13.10±9.49**	9.20±1.04	10.18±1.25	10.90±1.88	12.52±1.22*
Xanthoria parietina (L.) Bett.	slans	3.90±0.86	9.19±1.34*	7.20±0.90	6.06±0.34	5.23±0.29	6.68±0.92	9.05±1.75*	11.34±1.16**

Note: * – $p < 0.05$, ** – $p < 0.01$ in comparison with the control (medium without Fluconazole)

[8], strains with MFC ≤ 2 μg/ml were considered to be sensitive to Fluconazole. Both of efflux pumps inhibitors effectively reduced *C. tropicalis* strain resistance to Fluconazole virtually to the breakpoint. This provided an opportunity to conclude that this strain resistance to Fluconazole was provided mainly by the efflux mechanism. *C. albicans* strain MFC to Fluconazole decreased 8-fold in the presence of chlorpromazine subinhibitory concentrations and only 2-fold in the presence of Haloperidol. In case of both combinations, Fluconazole MIC

was significantly higher than the threshold for susceptible strains according to EUCAST criteria. Therefore, assumptions can be made about the dual nature of *C. albicans* strain azole resistance, namely the combination of the efflux mechanism of resistance with mutation(s) of ergosterol biosynthesis enzymes.

A very sensitive agar diffusion micromethod was used in order to differentiate extracts with high, weak and questionable antimicrobial activity. The same method was adapted for extracts screening for synergistic interaction with Fluconazole. Similar experiments were duplicated on media with subfungistatic antifungal concentrations ($^1/_4$, $^1/_8$ or $^1/_{32}$ MFC for each test strain). This method is definitely less sensitive than the checkerboard titration method or analysis of the dynamics of cultures growth curves. However, it provides an opportunity to test simultaneously a large number of drugs to discriminate them according to their activity and select the most promising of them for further research.

114 extracts out of 166 studied ones (68.7 ± 0.28%) showed direct antifungal activity in relation to *C. tropicalis* strain, 74 extracts (44.6 ± 0.30%) turned out to be highly active (d IZ > 10 mm). Only 50 extracts out of 166 studied ones (30.1 ± 0.28%) showed antifungal activity against *C. albicans strain*, 26 extracts (15.7 ± 0.22%) were highly active (d IZ > 10 mm). Significant direct antifungal activity both against *C. albicans* strain and *C. tropicalis* strain was demonstrated by the extracts of the leaves of *Sophora japonica*, thallus of *Mnium cuspidatum Hedw. (M.silvaticum Lindb.)*, herbs *of Euphorbia amygdaloides L., Lathyrus niger (L.) Bernh., Betonica officinalis L. s. l.,* flowers of *Primula officinalis Hill.,* roots of *Scrophularia nodosa L*.

The diameters of IZ of fungal cultures around the holes with a number of extracts were significantly larger than in control experiments (in a medium without antifungal agents) when testing plant extracts in media with subfungistatic concentrations of Fluconazole. This may indicate the presence of compounds capable of modifying the *Candida* resistance to Triazole antifungals, in particular Fluconazole, in the respective extracts. When screening for synergism with fluconazole, out of 166 studied extracts, 23 of them showed the most interesting results presented in the Table III. 26 extracts (15,7±0,22%) showed highly synergism activity against *C. tropicalis* strain with efflux mechanism of resistance to Fluconazole which is more susceptible to modification by biologically active compounds of medicinal plants than the resistance of the combined type in *C. albicans* test strain – 8 extracts (4,8±0,13%). In our opinion, representatives of *Lamiaceae* family plants, the family *Euphorbiaceae*, the family *Rosaceae* should be paid special attention when searching for modifiers of *Candida* resistance to Fluconazole. The ability to increase sensitivity of both test strains to Fluconazole was demonstrated by extracts of inflorescences of *Calendula officinalis L.*, *Rudbeckia laciniata L.*, aerial part of *Melilotus albus Medik*., aerial part and roots of *Agrimonia eupatoria L.*, herbs of *Thymus serpyllum L.*, leaves of *Juglans regia L.*, thallus of *Xanthoria parietina (L.) Bett*.

DISCUSSION

Medicinal plants are an extremely rich natural source of biologically active compounds, including those with antifungal properties [4]. Considering the growing resistance of *Candida* fungi to existing antifungals and their extremely limited array, the combination therapy consisting in the combined use of synergistic agents is a very promising direction in managing opportunistic candidiasis. A number of phytocompounds (primarily phenylpropanoids, flavonoids and terpenoids) can be described as modifiers of antifungal resistance [3,4], which are able to neutralize the mechanisms of acquired resistance to classical antifungal agents. Alkaloids (berberine, tetrandrin), flavonoids (baicalein, kaempferol [10], quercetin), terpenoids (thymol, carvacrol [11, 13], farnesol [12], eugenol [14,13], menthol, menthone, carvol) as well as magnolol, honokiol, curcumin [15], methylcyamaldehyde, allyl isothiocyanate shows synergism with Fluconazole. Our research shows that modifiers of *Candida* resistance to Fluconazole can be widely represented in medicinal plants of the Ukrainian flora, and this issue requires further in-depth study, as it may be of great practical importance. It should be noted that such flavonoids as quercetin and kaempferol, as well as terpens are extremely common in plant materials.

In general, the results of large-scale screening provide valuable information about the new type of action (an ability to increase the sensitivity of *Candida* strains resistant to Fluconazole) of a number of officinal and nonofficial medicinal and aromatic plants. In the longer term, they can become a raw material for the creation of new antifungal drugs and increase the effectiveness of antifungal therapy. The presented results can be considered encouraging taking into account the urgency and unresolved issue of effective antifungal therapy of opportunistic infections caused by antifungal-resistant strains of *Candida* fungi. They are aimed at directing further research in a more rational direction and they may be the basis for the implementation of combination antifungal therapy in clinical practice over time. An increase in the dosage or combination of two antifungal drugs cannot solve the problem, since this leads to increased side effects or toxicity. Combination therapy

is more beneficial than monotherapy because it may provide more effective destruction or suppression of pathogens. The combined action of synergistic agents is quite easy to achieve in case of localized superficial lesions (which occur on the mucous membranes of the prosthetic bed in candidal denture stomatitis): for example, using a systemic antifungal agent and a topical agent that contains a modifier of resistance of plant origin fungi. Synergistic interactions will increase the therapeutic efficacy of drugs, reduce the likelihood of resistance occurrence or further development, and will reduce dose-related toxicity.

CONCLUSIONS

1. Aqueous-ethanolic extracts of medicinal and aromatic plants of Ukrainian flora have direct antifungal activity against azole resistant *C. albicans* and *C. tropicalis* (44,6±0,30% and 15,7±0,22% of tested extracts respectively) associated with denture stomatitis as well restore their sensitivity to fluconazole (44,6±0,30% and 15,7±0,22% of extracts respectively).
2. The efflux mechanism of Fluconazole resistance in *C. tropicalis* test strain is more susceptible to modification by biologically active compounds of medicinal plants (15,7±0,22% of extracts showed significant synergism) than the resistance of the combined type in *C. albicans* test strain (4,8±0,13% synergic extracts).
3. The ability to increase the sensitivity of both *Candida* test strains to Fluconazole was demonstrated by the inflorescences of *Calendula officinalis L.*, *Rudbeckia laciniata L*, aerial part of *Melilotus albus Medik*., aerial part and roots of *Agrimonia eupatoria L.*, herb of *Thymus serpyllum L*., leaves of *Juglans regia L*., thallus of *Xanthoria parietina (L.) Bett*.
4. Plants of the families Lamiaceae, Euphorbiaceae, and Rosaceae are the most promising for the search for inhibitors of *Candida* Fluconazole efflux pumps.

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ORIGINAL ARTICLE

PARAMETERS OF LEFT VENTRICULAR DIASTOLIC DYSFUNCTION IN PATIENTS WITH HYPERTENSION DISEASE WITH CONCOMITANT TYPE 2 DIABETES

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ABSTRACT

The aim: To study the parameters of the left ventricular (LV) diastolic function in patients with HT with concomitant T2DM and without it before and after complex treatment with the inclusion of Eplerenone 50 mg per day and Trimetazidine 80 mg per day during 3 months.

Materials and methods: The study included 50 patients, aged 45–54 years (mean age 51.3±1.5 years), women – 24 and men 26 with HT stage II. All patients were divided into 2 groups: 1 group (n=25) – patients with HT stage II (HbA1c level of 5.01±0.13%) and 2 group (n=25) – patients with HT stage II and concomitant T2DM (HbA1c level of 7.6±0.34%). The control group consisted of 20 healthy individuals (HbA1c level of 4.68±0.49%).

Results: When analyzing the findings on left atrial volume index (LAVI), the highest indicators were observed in patients with HT with T2DM, but slightly lower in HT, and even lower in the control group, but the differences at this stage were not significant. This suggests that functional changes in cardiomyocyte kinetics, which develop in patients with comorbid pathology and are caused by metabolic and hemodynamic disorders, can progress steadily.

Conclusions: After a three-month course of treatment with Eplerenone and Trimetazidine, the rate of myocardial relaxation in diastole likely increased in both groups of those examined. The prescribed treatment with Eplerenone and Trimetazidine has led to a decrease in the rate of progression of heart failure and a reduction in cardiovascular risks.

KEY WORDS: hypertension, type 2 diabetes mellitus, Eplerenone, Trimetazidine, diastolic flow, comorbid pathology

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INTRODUCTION

Nowadays, hypertension (HT) remains the most widespread non-communicable pandemic in human history, which causes cardiovascular morbidity and mortality. [1] Cardiovascular mortality holds a leading position in the overall mortality of the world's population. This is attributed to the high comorbidity of cardiovascular pathology with other diseases. [2,3] The correlation between HT and various pathological conditions, which largely determine its progression and development of cardiovascular complications, is evident and well-proven. One of these conditions or diseases is type 2 diabetes mellitus (T2DM), with about 400 million patients worldwide, which is projected to increase to 700 million by 2030. [4,5] The aggregate impact of HT and T2DM greatly impairs the quality of life of patients and leads to increased disability and fatal cardiovascular outcomes. [6,7]

Comorbid patients are becoming more prevalent in modern clinical practice, which brings certain difficulties in the diagnosis and treatment of such patients [8-10]. Timely diagnosis and treatment of cardiovascular complications in T2DM patients in the early stages refer to important tasks both from the point of view of prevention and improvement of the course of comorbidities. T2DM dramatically reduces both the quality of life and its average duration. The reason for such an adverse effect of T2DM consists in the alterations it causes in the cardiovascular system, kidneys, and other systems. Alterations in the cardiovascular system in the diabetic population can trigger significant disorders in various organs and systems of the body and have a negative mutual influence. [7]

Timely diagnosis and treatment of cardiovascular and nephrological alterations in diabetic patients in the early stages are among the major tasks both from the point of view of prevention and improvement of the course of comorbidities. [7]

THE AIM

To study the parameters of the left ventricular (LV) diastolic function in patients with HT with concomitant T2DM and without it before and after complex

treatment with the inclusion of Eplerenone 50 mg per day and Trimetazidine 80 mg per day during 3 months.

MATERIALS AND METHODS

The study was conducted according to the main provisions of the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Declaration of Helsinki of the World Medical Association with an overview of the ethical principles for medical research involving human subjects (1964-2004), the requirements of Good Clinical Practice (GCP) from 1996, and the Order of the Ministry of Health of Ukraine No. 690 as of 23.09.2009.

The study included 50 patients, aged 45–54 years (mean age 51.3±1.5 years), women – 24 and men 26 with HT stage II. All patients were divided into 2 groups: 1 group (n=25) – patients with HT stage II (HbA1c level of 5.01±0.13%) and 2 group (n=25) – patients with HT stage II and concomitant T2DM (HbA1c level of 7.6±0.34%). The patients were comparable in terms of age, sex, duration of the disease on HT, they were being treated at the clinic of the GI "L.T. Malaya Therapy National Institute of the NAMS of Ukraine". The control group consisted of 20 healthy individuals (HbA1c level of 4.68±0.49%). All patients were examined in accordance with the recommendations of the European Society of hypertension and the European Society of Cardiology (ESH/ESC, 2019). All respondents signed an informed consent to participate in the study. The diagnosis of T2DM was established according to the recommendations of the American Diabetes Association (ADA, 2020)

The inclusion criteria were patients with HT stage II and patients with HT stage II and concomitant T2DM. The non-inclusion criteria were patients with type 1 diabetes mellitus, congenital heart, and urinary tract defects, presence of an artificial pacemaker, presence of artificial heart valves, heart failure stage II B and III, acute myocardial infarction, infectious and severe inflammatory processes, hematological and oncological diseases.

All patients and control subjects underwent general clinical and laboratory examination (clinical and biochemical blood and urine tests, albuminuria, carbohydrate metabolism, etc.), electrocardiography (ECG), and anthropometric measurements.

Research hypothesis is a positive effect of treatment with Eplerenone and Trimetazidine for 3 months on the state of cardiohemodynamics is expected.

The diastolic function of the heart was assessed by transthoracic echocardiography, which was performed on the ULTIMA PA ultrasound machine ("Radmir", Ukraine) using a phased array transducer with a frequency range of 2-3 MHz according to the standard method of the American Society of Echocardiography [11].

To assess the LV diastolic function by pulsed-wave Doppler echocardiography, transmitral blood flow parameters were determined: E/A ratio (where E is the peak flow velocity of the early filling period, and A is the peak flow velocity of the late filling period). Furthermore, according to the guidelines of the American Society of Echocardiography and the European Association of Cardiovascular Imaging in 2016, the left atrial volume (LAV), left atrial volume index (LAVI) were measured using tissue Doppler in pulsed-wave mode, and the mean value of the early diastolic velocity of the fibrous ring (e`mean) was calculated [11-12]. E/e` (the ratio between the transmitral flow velocity and the average velocity of the fibrous ring of the mitral valve) was calculated as well. This indicator reflects indirectly the filling pressure of the left ventricle [13-15].

Statistical analysis of the data was performed using Statistica, 8.0 (Stat Soft Inc, USA), Microsoft Office Excel 2003. The Kolmogorov-Smirnov test was used to assess the nature of the aggregate distribution of the sample data. Between-group differences in mean values and their errors (M ± m) were evaluated using the Student's t-test. A probable error of less than 5 % ($p < 0.05$) was considered reliable.

The main parameters of transmitral blood flow were analyzed to determine the LV diastolic function in patients with HT and T2DM and HT: E, A, E/A, kinetics parameters of the fibrous ring of the mitral valve: early diastolic velocity e' of the lateral (e`lateral) and septal (e`septal) parts and calculating the mean value of the early diastolic velocity of the fibrous ring (e`mean); LAV, LAVI. The ratio between the transmitral flow velocity and the average velocity of the fibrous ring of the mitral valve E/e` was calculated, which reflects indirectly the filling pressure of the left ventricle.

RESULTS

The data in Table I suggest that in patients with HT and HT with comorbid T2DM, the mean kinetics of diastolic flow of the fibrous ring was significantly lower in comparison with the control group. While the e`mean level in patients with T2DM and HT was significantly lower in comparison with patients with HT. Thus, the findings indicate that aggregate hemodynamic and metabolic alterations adversely affect the kinetic capabilities of the myocardium. Moreover, the lowest rates of the myocardial diastolic relaxation rate were probably found in patients with HT with T2DM. The latter proves that the comorbidity of these negative factors significantly reduces the functional capacity of the myocardium.

Table I. Indicators of LV diastolic function in patients with HT and type 2 DM before treatment (M±m)

Indicators	HT and T2DM n = 25	2 grade HT n = 25	Control n = 20
E, cm/s	59.91±5.42*	64.81±6.25	77.44±5.04
A, cm/s	82.05±4.38*	67.48±5.48*	55.11±4.63
E/A	0.73±0.15*	0.99±0.32*	1.44±0.16
LAV, ml	54.83±6.86*	58.33±5.31*	44.00±3.83
LAVI, ml/m²	29.33±2.05*	26.31±1.84	23.63±1.65
e`mean, cm/s	7.76±1.33	8.98±1.44	14.26±1.90
E/e`mean	7.88±2.16*	7.14±1.44	6.07±1.16

Note. *Significant difference in indicators compared to the control, $p < 0,05$

Table II. Indicators of LV diastolic function in patients with HT and type 2 DM after treatment (M±m)

Indicators	HT and T2DM n = 25	2 grade HT n = 25	Control n = 20
E, cm/s	59.91±5.42	64.81±6.25	77.44±5.04
A, cm/s	82.05±4.38	67.48±5.48	55.11±4.63
E/A	0.73±0.15	0.99±0.32	1.44±0.16
LAV, ml	52.83±6.86	49.33±5.31	44.00±3.83
LAVI, ml/m²	28.91±1.95*	26.31±1.84	23.63±3 .91
e`mean, cm/s	8.64±1.33*	9.92±1.44	14.26±1.90
E/e`mean	7.23±2.16	6.77±1.44	6.07±1.16

Note. *Significant difference in indicators compared to the control, $p < 0,05$

One of the key criteria for assessing LV diastolic function is the ratio of the peak flow velocity of the early filling period E to the mean value of the early diastolic velocity of the fibrous ring of the mitral valve e`mean (E/e`mean reflects indirectly the increase in pressure in the LV cavity).

An increase in parameters indicating elevated pressure in the LV cavity may result in changes in the volumetric parameters of the left atrium (LA). Therefore, the next major parameter in the analysis of diastolic dysfunction, which may indicate the development of diastolic heart failure, is an increase in left atrial volume (LAV). Thus, a significant increase in the left atrial volume was found in both groups (HT and HT with T2DM) compared to the control group ($p<0.05$), while this indicator did not differ significantly between the groups.

When analyzing the findings on left atrial volume index (LAVI), the highest indicators were observed in patients with HT with T2DM, but slightly lower in HT, and even lower in the control group, but the differences at this stage were not significant. This suggests that functional changes in cardiomyocyte kinetics, which develop in patients with comorbid pathology and are caused by metabolic and hemodynamic disorders, can progress steadily. Eventually, these changes may progress to dystolic and systolic dysfunction and further worsening of heart failure.

The analysis of LV diastolic function parameters after a three-month treatment with Eplerenone 50 mg per day and Trimetazidine 80 mg per day revealed positive changes in LV hemodynamic parameters (Table II).

Table II shows that the mean kinetics of diastolic flow of the fibrous ring after treatment were significantly lower in the group of patients with HT and HT with compared with the control group. While the e`mean level in patients with HT and T2DM was significantly lower compared with patients with HT. After treatment, the myocardial diastolic relaxation rate was significantly accelerated in both groups (HT and HT with T2DM) compared with pre-treatment ($p<0.05$) (Table II).

Thus, the myocardial kinetic capabilities after treatment have significantly improved, indicating the possibility of reverse shifts in the functional state of cardiomyocytes at this stage of disease and the absence of significant fibrosing alterations, which are leveled by compensatory capabilities when hemodynamic and metabolic disorders of the myocardium are normalized.

It is worth noting that the absolute values of the E/e`mean ratio, (LAV), and (LAVI) after a three-month treatment with Eplerenone and Trimetazidine became lower compared to their pre-treatment values. Our findings are consistent with the results of other researchers [16-19].

Thus, a three-month treatment with Eplerenone and Trimetazidine contributed to the normalization of hemodynamics and metabolic processes, led to an improvement in myocardial functionality (increased myocardial diastolic relaxation rate), inhibition or termination of further development of adverse changes in cardiohemodynamics such as an increase in left ventricular filling pressure (E/e`mean) and impaired geometric parameters of the heart (LAV).

DISCUSSION

Analyzing the results for indexed left atrial volume (iLAV), it should be stated that the highest rates were in patients with HT and T2DM, somewhat lower at HT, and even lower in the control group, but the differences didn't reach a probable level. This suggests that functional changes in cardiomyocyte kinetics that occur in patients with comorbid pathology and are caused by metabolic and hemodynamic disorders, can progress relentlessly. Ultimately, these changes are transferred into diastolic and systolic dysfunction, and into further progression of heart failure.

One of the most significant criteria for evaluating the diastolic function of LV is the ratio of the maximum flow rate of the early filling period E to the mean value of the rate of early diastolic movement of the fibrous ring of the mitral valve e'mean (E/e'mean indirectly reflects the increase in pressure in the LV cavity).

As the results show, a significant increase in the ratio of E/e'mean occurs in the group of patients with HT and T2DM and HT (p). In this case, the probable difference in the ratio of E/e'mean between the HT and HT groups with DM Type 2 is not established. However, the highest indicators of this ratio (E/e`mean) were found in patients with HT and T2DM. As a result, an increase in the parameters that indicate an increase in pressure in the LV cavity can be changes in the volume indicators of the left atrium (LA). Therefore, the next important parameter in the analysis of the state of diastolic dysfunction, which may indicate the development of diastolic heart failure is an increase in left atrial volume (LAV). Thus, a significant increase in left atrial volume in both groups (HT and HT with T2DM) ($p<0.05$) was found compared to the control group, while this indicator probably didn't differ between groups.

The analysis of the kinetic capabilities of the myocardium after treatment indicates a significant improvement in them, which indicates the possibility of reverse shifts in the functional state of cardiomyocytes at this stage of the disease and the absence of significant fibrosing changes that level out the compensatory capabilities in the normalization of hemodynamic and metabolic disorders of the myocardium.

It is noteworthy that the absolute ratios of E/e'mean, (LAV) and (iLAV) after three months of treatment with eplerenone and trimetazidine were lower compared to their pre-treatment values. The data we obtained are consistent with the results of other researchers [16-19].

Thus, three-month treatment with eplerenone and trimetazidine contributed to the normalization of hemodynamics and metabolic processes, led to an improvement in myocardial functionality (an increase in the rate of myocardial relaxation in diastole), inhibition or cessation of further development of adverse changes in cardiohemodynamics in the form of an increase in the pressure of filling the left ventricle (E/e'mean) and a violation of the geometric parameters of the heart (LAV).

Therefore, the study of the parameters of the diastolic function of the left ventricle at the stage of functional myocardial damage is necessary in order to prevent or reverse the development of heart failure in patients with comorbid pathology (HT with T2DM), which is the key to improving cardiovascular prognosis.

CONCLUSIONS

1. In patients with hypertensive disease of the II stage and hypertensive disease with concomitant type 2 diabetes mellitus, the values of the kinetics of the diastolic movement of the fibrous ring of mitral valve were significantly lower compared to similar indicators of the control group, and these changes were more expressive in comorbid patients.
2. After a three-month course of treatment with Eplerenone and Trimetazidine, the rate of myocardial relaxation in diastole likely increased in both groups of those examined.
3. The prescribed treatment with Eplerenone and Trimetazidine has led to a decrease in the rate of progression of heart failure and a reduction in cardiovascular risks.

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ORIGINAL ARTICLE

DEVELOPMENT OF A MATHEMATICAL MODEL OF SELECTING THE EXTENT OF A SURGICAL INTERVENTION IN SPINAL TUMOR

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ABSTRACT

The aim: To develop a mathematical model of selecting the extent of surgical intervention in the spinal tumors.

Materials and methods: The retrospective study included 237 patients with spinal tumors who underwent the following surgeries: vertebroplasty (V); vertebroplasty and spinal fixation (F+V); posterior spinal decompression and spinal fixation (F+F); vertebrectomy and replacement of vertebra by a cage with posterior spinal fixation (F+F+K). The mathematical model is based on the modified Spine Instability Neoplastic Score (SINS). The patients were divided into two clusters. Cluster analysis was used to build a diagnostic decision tree model.

Results: The difference between two clusters is determined by the extent of surgical intervention, the grade of the vertebral lesion, epidural compression, and local kyphosis, and neurological signs as well. The cluster 1 included 115 patients with higher values of SINS compared to the cluster 2. All cases of vertebroplasty belonged to the cluster 2. In the cluster 1 cases of surgery of large extent: F+F; F+V; F+F+K. Analysis of the decision tree model for cluster 1 showed that a type of surgery was determined for 97 patients from 115 that relates to 84.3% of overall accuracy. The decision tree model have a high predictive accuracy for the surgery F+V and better indicators of coverage and predictive accuracy for the surgery F+F+K.

Conclusions: Our study developed a decision tree model to optimize spinal neoplasm surgery, achieving 84.3% accuracy based on significant prognosis criteria. The model considers surgical type, neurological signs, vertebra lesion grade, and stage of epidural compression, potentially improving clinical outcomes.

KEY WORDS: prognosis, cluster analysis, decompression, decision tree, spinal neoplasm

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INTRODUCTION

Surgery has a leading role in spinal tumor treatment. It can improve mechanical stability, lead to nerve decompression, and reduce the amount of pain [1, 2]. Decompression techniques used in the past, without stabilization, often led to negative results, and many specialists took the view that radiation therapy was the best option for treating patients with spinal tumor than surgery was [3, 4]. However, achievements analysis in modern surgery has shown that stabilizing operations in multimodality therapy allow achieving good results [4–6]. It is noteworthy that the morbidity rates after surgical interference may be as high as 20-30% [7, 8]. This is especially true for en block-based big resections, relate to a high complexity compared to palliative care [9–11].

Integration of modern diagnostic techniques makes it possible to choose an individual approach to the treatment of each patient, depending on the type and neoplasm location. Prognostic and diagnostic scales are used in clinical practice to improve treatment outcomes that help to select a balanced tactic [2, 12–16].

There are still discussions on the advantages and disadvantages of their use, despite the satisfactory level of sensitivity and specificity of the available diagnostic algorithms [17]. The classifications and rating scales that are currently used in clinical practice, do not fully determine which surgical intervention should be performed in a particular patient, providing only general advice for treatment [15, 18]. Therefore, we believe that it is advisable to improve the known and develop novel algorithmic approaches to spinal tumor treatment.

THE AIM

The aim of study was to develop a mathematical model of selecting the extent of surgical intervention in the spinal tumors.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board (Protocol No. 164 dated 18 April 2017). All patients signed informed consent forms for participating in the study.

PATIENTS

The retrospective study included 237 patients (127 women and 110 men) with oncological alterations in the spine who received one of four type's surgical treatment at the Department of the Spine Diseases and Injuries of Institute from 2008 to 2017 (Table I). The age of the patients was 18–89 years, body mass index varied from 18.0 to 21.5 kg/m^2. The patients were divided into 4 groups on the extent of surgical intervention according to their medical charts: vertebroplasty or kyphoplasty (V); vertebroplasty including transpedicular fixation of the spine one vertebra above and below (F+V); posterior spinal decompression and stabilization of two vertebrae above and below the decompression zone (F+F); vertebrectomy and replacement of vertebra by a cage with an additional stabilization of the spine by implant (F+F+K).

PATIENT ASSESSMENT SCALE

The mathematical model is based on the SINS Neoplastic Spine Instability Scale [12], which was supplemented with the following indicators: neurological examination assessment on the ASIA scale [19]; type of tumor that requires palliative/radical surgery; degree (stage) of epidural compression according to the M. H. Bilsky [14]; the size of local kyphosis; the existence of adjacent vertebrae destruction (Table II). All of the above characteristics are considered in practice when deciding on the tactics of surgical management of neoplasms of the spine and according to expert view, they preliminarily assigned the scores. According to the developed system, the increase of the number of scores should reflect an increase of the extent of surgical intervention. The proposed scoring system that considers nine indicators was tested on a sample.

STATISTICAL METHODS

The Kruskal-Wallis test was used to compare four groups of patients with different types of surgery, and the Mann-Whitney test was used to compare two groups. Nominal data were given as medians (Me) and interquartile range [LQ; UQ], minimum and maximum sample (Min; Max). Cluster analysis (k-means clustering) was used to build a diagnostic decision tree. To compare the occurrence of certain signs between two clusters of patients, the Z-test was used. Chi-square test was used to identify the influence of potential predictive indicators in cluster 1. The obtained results were used to build a diagnostic decision tree model on the choice of surgical intervention. A critical level of significance was accepted as 0.05. The analysis was performed using the Statistica 12 (TIBCO Software Inc., USA).

RESULTS

The hypothesis on an increase of the scores in the proposed modified scale SINS (Table II) coheres with an increase in the extent of surgical intervention (Kruskal-Wallis test; $H=182.7176$; $p\leq0.05$). The scores between all four patients' groups were significantly different (Table 3). However threshold values of the scores which can be used to determine the acceptable accuracy of the specific extent of the surgery was not established. During the testing of the linear rating scale, the best accuracy of determining the extent of surgery was obtained by applying the rules with the following threshold scoring values: a) less 10 –"V"; b) from 10 to 13 – "F+V"; c) from 14 to 16 – "F+F"; more than 16 – "F+F+K".

Two clusters of patients were identified by used cluster analysis. The patients in the clusters were similar in these indicators as the presence of the destruction of adjacent vertebrae, type of bone lesion, type and tumor location. The difference between clusters is determined by the extent of surgical intervention, the grade of the vertebral lesion, epidural compression, and local kyphosis, and neurological signs as well. The cluster 1 included 115 patients with higher values of these indicators compared to 122 patients who were assigned to the cluster 2 (Table IV).

Further analysis of the structure of the obtained clusters showed that cluster 1 corresponds to symptoms of a greater severity and a higher severity of the patient (Table IV). The lesions located in spinal segments with the limited movement were observed equally frequently in both clusters; those within the interjacent spine region were more frequently observed in the cluster 1 ($p=0.002$) and those in the movable spinal unit were observed in the cluster 2 ($p=0.006$) (Table IV).

Lytic spinal bone lesions were detected in 93.9% in the cluster 1, a significantly higher compared to 76.2% of such cases in the cluster 2 ($p<0.000$). At the same time, the proportion of the patients with mixed type bone lesion was significantly higher in cluster 2 than in the cluster 1 ($p=0.001$) (Table IV).

In particular, the cluster 2 did not contain the individuals with a local kyphosis of more than 21°. The patients with a local kyphosis between 13° and 21° were in both clusters, but their proportion was significantly higher in the cluster 1

Table I. Distribution of patients according to diagnosis

Primary tumor (n=110)	n	Spinal metastatic change (n=127)	n
Plasma cell myeloma (multiple myeloma)	31	Poorly differentiated cancer	28
Hemangioma	17	Adenocarcinoma	24
Giant cell tumor	12	Undifferentiated cancer	24
Eosinophilic granuloma	10	Breast cancer	14
Malignant lymphoma	9	Clear cell renal cell carcinoma	13
Chordoma	5	Squamous cell cancer	11
Aneurysmal bone cyst	5	Granular cell cancer	5
Chondrosarcoma	3	Thyroid cancer	4
Osteoid osteoma	3	Seminoma	1
Osteoblastoma	3	Malignant chemodectoma	1
Fibrosarcoma	3	Melanoma	1
Osteosarcoma	2	Small cell lung cancer	1
Osteochondroma	2		
Angiosarcoma	2		
Ewing's sarcoma	1		
Polymorphcellular sarcoma	1		
Malignant fibrous histiocytoma	1		

Table II. Modified Spine Instability Neoplastic Score (SINS)

№	Component	Score	№	Component	Score
1	Location		6	Neurological signs (ASIA scale):	
	interjacent spine region (nape–C2, C7–Th1, Th12–L1, L5–S1);	3		A	2
	movable spinal unit (C2–C6, L2–L4);	2		B	2
	spinal segments with limited movement (Th3–Th10);	1		C	2
	immovable spinal segment (S2–S5)	0		D	1
2	Pain			E	0
	at exertion and rest;	2	7	Stage of epidural compression:	
	only at exertion;	1		stage III, Bilsky 3;	3
	only at rest	0		stage II, Bilsky 2, Bilsky 1c;	2
3	Rachiopathy (bone destruction)			stage I, Bilsky 1b, Bilsky 1a;	1
	lytic;	2		stage 0, Bilsky 0	0
	mixed (lytic/blastic);	1	8	Type of surgery(depending on the type of tumor):	
	blastic	0		radical;	3
4	Spine shape (size of local kyphosis)			palliative	0
	> 30°;	3	9	Destructive of adjacent vertebrae:	
	22°–30°;	2		absent;	0
	13°–21°;	1		presented	1
	< 12°	0			
5	Grade of vertebral lesion				
	body collapse with destruction of the posterior backbone complex;	5			
	more than 60%, collapse;	4			
	31–60 %, collapse;	3			
	less than 30%, collapse;	2			
	without collapse	1			

Table III. Threshold score values on a modified scale (SINS) for patient groups based on extent of surgery

Group of patients	Score		Mann-Whitney test
	Me [LQ; UQ]	Min; Max	
V	8 [6; 9]	3; 14	V: U=52,5; p<0,01 F+F: U=17,0; p<0,01
F+V	12 [12; 14]	10; 15	F+F: U=113,5; p<0,01 F+F+K: U=66,5; p<0,01
F+F	16 [14; 16]	12; 20	F+F+K: U=632,0; p<0,01
F+F+K	17 [16; 19]	10; 23	V: U=35,0; p<0,01

Data presented as medians (Me) and interquartile range [LQ; UQ]; minimum and maximum sample (Min; Max). Group of patients: vertebroplasty or kyphoplasty (V); vertebroplasty with transpedicular fixation of the spine one vertebra above and below (F+V); posterior spinal decompression and stabilization of two vertebrae above and below the decompression zone (F+F); vertebrectomy and replacement of vertebra by a cage with an additional stabilization of the spine by implant (F+F+K).

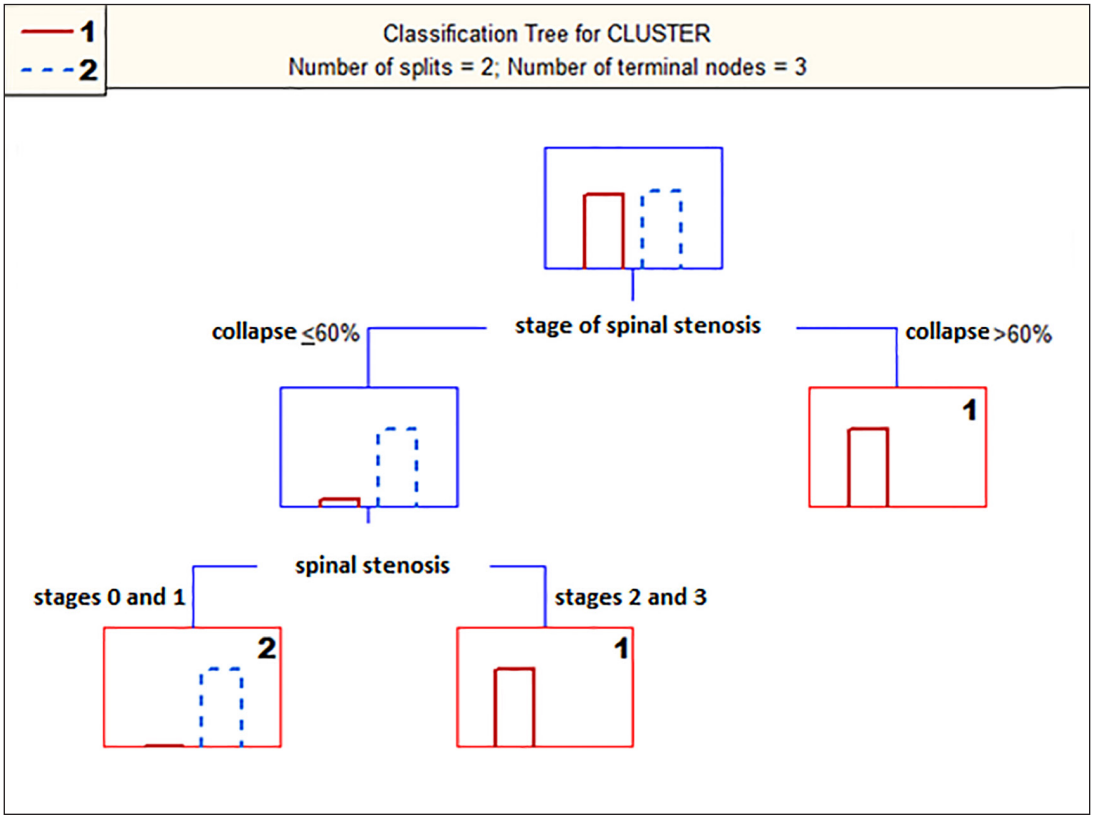

Fig. 1. Decision tree for identifying a patient cluster. Cluster 1 is a full line; the cluster 2 is a dashed line.

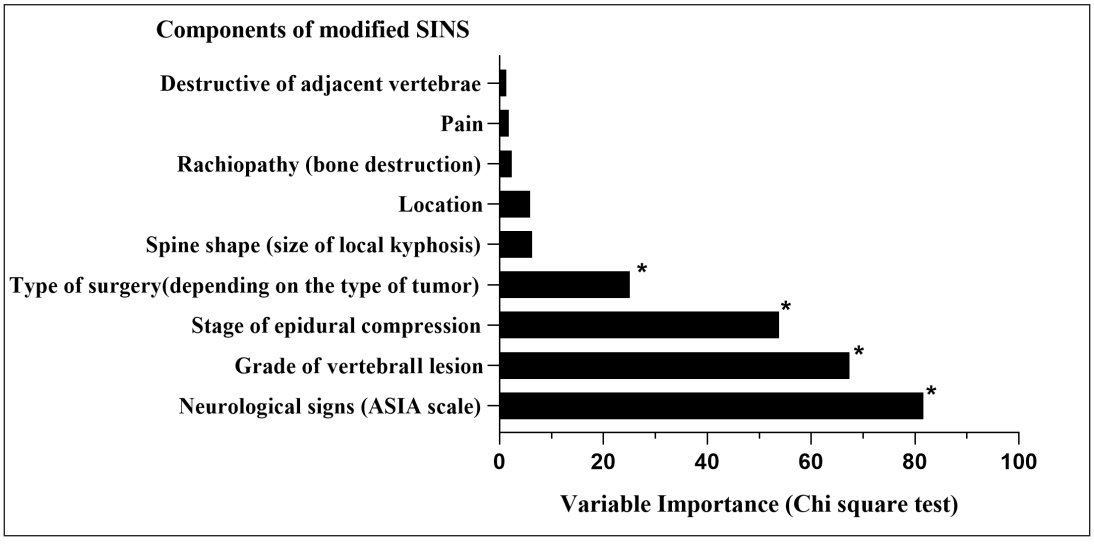

Fig. 2. Result of determining the importance components of the modified SINS for extent choice of surgical intervention in the cluster 1. Chi-square test. *p<0.000.

Table IV. Assignment of the patients by two clusters (n, %) in Modified Spine Instability Neoplastic Score (SINS)

	Component	Patients		Z-test
		Cluster 1 (n=115)	Cluster 2 (n=122)	
		n (%)	n (%)	
1	Location			
	interjacent spine region (nape–C2, C7–Th1, Th12–L1, L5–S1)	70 (60.9)	52 (42.6)	Z=2.858, p=0.002
	movable spinal unit (C2–C6, L2–L4)	27 (23.5)	47 (38.5)	Z=2.542, p=0.006
	spinal segments with limited movement (Th3–Th10)	18 (15.6)	23 (18.9)	Z=0.653, p=0.256
2	Pain			
	at exertion and rest	75 (65.2)	42 (34.4)	Z=4.980, p<0.000
	only at exertion	40 (34.8)	60 (49.2)	Z=2.270, p=0.012
	only at rest	0	20 (16.4)	—
3	Rachiopathy (bone destruction)			
	lytic	108 (93.9)	93 (76.2)	Z=3.972, p<0.000
	mixed (lytic, blastic)	7 (6.1)	23 (18.9)	Z=3.051, p=0.001
	blastic	0	6 (4.9)	—
4	Spine shape (size of local kyphosis)			
	> 30°;	5 (4.3)	0	—
	22°–30°	52 (45.2)	0	—
	13°–21°;	47 (40.9)	18 (14.8)	Z=4.666, p<0.000
	< 12°	11 (9.6)	104 (85.2)	Z=17.922, p<0.000
5	Grade of vertebral lesion			
	body collapse with destruction of the posterior backbone complex	51 (44.4)	0	—
	more than 60% collapse;	52 (45.2)	0	—
	31–60% collapse;	10 (8.7)	35 (28.7)	Z=4.109, p<0.000
	less than 30% collapse	2 (1.7)	48 (39.3)	Z=8.197, p<0.000
	without collapse	0	39 (32)	—
6	Neurological signs (ASIA scale):			
	A	14 (12.2)	0	—
	B	15 (13.0)	0	—
	C	18 (15.7)	0	—
	D	35 (30.4)	0	—
	E	33 (28.7)	122 (100)	—
7	Stage of epidural compression:			
	stage III, Bilsky 3;	38 (33.0)	0	—
	stage II, Bilsky 2, Bilsky 1c;	61 (53.0)	0	—
	stage I, Bilsky 1b, Bilsky 1a;	15 (13.0)	43 (35.2)	Z=4.154, p<0.000
	stage 0, Bilsky 0	1 (1.0)	79 (64.8)	—
8	Type of surgery (depending on the type of tumor):			
	radical;	22 (19.1)	0	Z=4.551, p<0.000
	palliative	93 (80.9)	121 (98.4)	
9	Destructive of adjacent vertebrae:			
	absent	88 (76.5)	108 (88.5)	Z=2.453, p=0.007
	presented	27 (23.5)	14 (11.5)	
10	Extent of surgery:			
	V	0	122 (100)	—
	F+V	18 (15.6)	0	—
	F+F	47 (40.9)	0	—
	F+F+K	50 (43.5)	0	—

Extent of surgery: vertebroplasty or kyphoplasty (V); vertebroplasty with transpedicular fixation of the spine one vertebra above and below (F+V); posterior spinal decompression and stabilization of two vertebrae above and below the decompression zone (F+F); vertebrectomy and replacement of vertebra by a cage with an additional stabilization of the spine by implant (F+F+K).

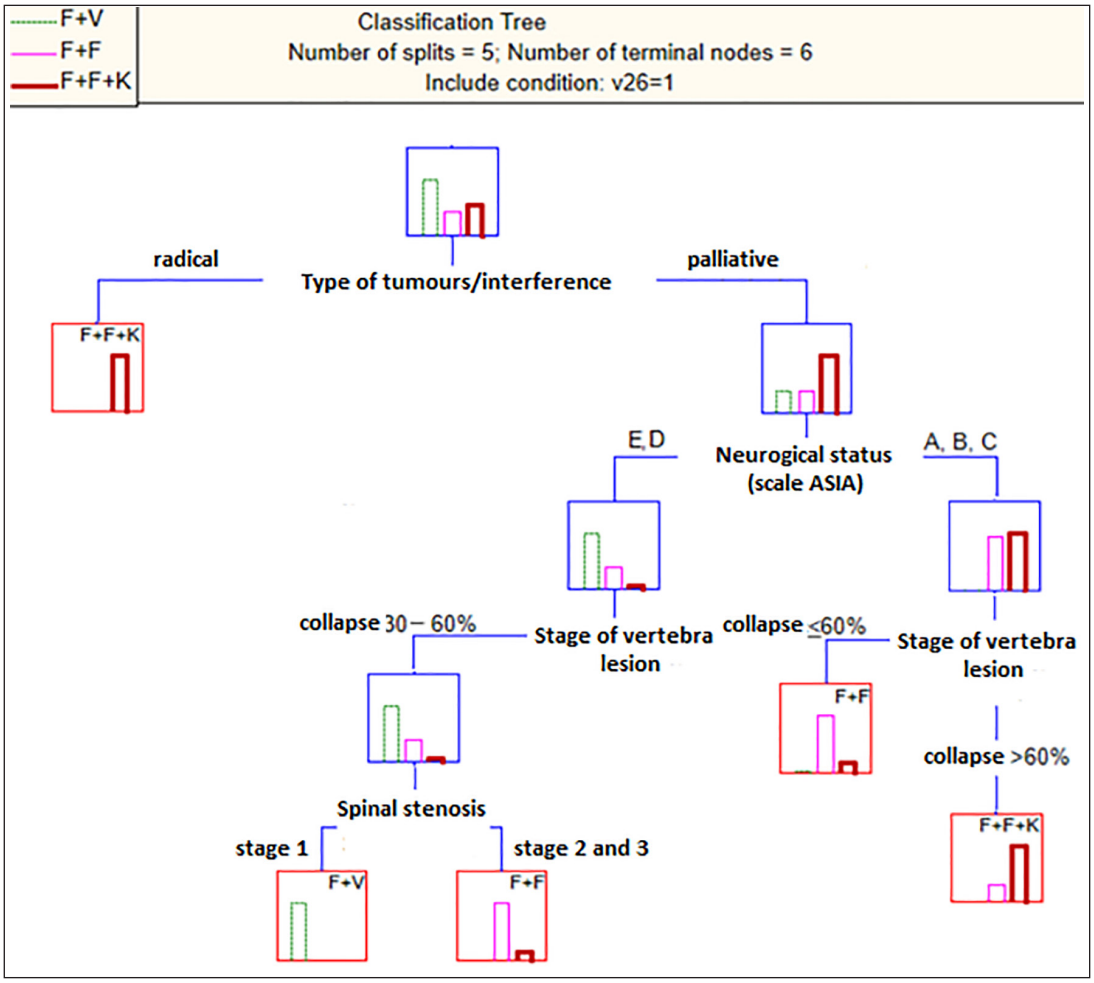

Fig. 3. Decision tree model for the choice of types of surgical intervention in the cluster 1.

(p<0.000). The majority of the patients with a local kyphosis less than 12° belonged to the cluster 2 (Table IV).

All cases of vertebral lesions with a body collapse and destruction of the posterior column and collapse of more than 60% belonged to the cluster 1, and all cases of vertebral lesions without collapse belonged to the cluster 2. The number of patients with a vertebral body collapse of less than 30% in cluster 1 was significantly lower compared to cluster 2 (p<0.000) (Table IV).

All patients who did not have any neurological symptoms (E grade in the ASIA scale) were in the cluster 2, while the neurological status could meet even the most severe stages of motor and sensory spinal dysplasia in the cluster 1, the majority of patients felt pain both during exertion and at rest (Table IV).

The tumors that caused the performance of radical surgical intervention were in the cluster 1 (p<0.000) (Table IV).

The described patterns allowed determining the most informative combination of features positing that the overall sample of the patients is stratified into two clusters. The found rule can be represented as a decision tree (Fig 1), whereby the cluster 1 should include the individuals whose degree of vertebral lesion relates to the collapse on the level of more than 60% or vertebral body collapse with the destruction of posterior backbone complex; if the collapse is not more than 60% and the degree of epidural compression (spinal stenosis) of stage II or III.

We should pay attention to the patients' allocation in the obtained clusters in the extent of surgical intervention (Table IV). In particular, all cases where vertebroplasty was performed belonged to the cluster 2. The patients in the cluster 1 were those who underwent surgery of a large extent. Thus, we can formulate an algorithm that prescribes the surgeries with the lowest extent (vertebroplasty) to determine the cluster of the patients based on the decision tree (Fig 1). The indicators for such operations are spinal stenosis at stages 0 or I, where the vertebral lesion comes with body collapse at the level of no more than 60%.

Further studies were aimed at determining the components of the modified SINS, which statistically significantly affect the distribution of patients in the cluster 1 into three groups, depending on the extent of surgical intervention. Chi square test showed that these components were neurological signs (ASIA scale) (p<0.000), grade of vertebral lesion (p<0.000), stage of epidural compression (p<0.000) and type of surgery (depending on the type of tumor) (p<0.000). Other components did not have a significant effect (Fig 2).

Further, using the obtained significant components, a decision tree model was built, in which the type of surgery is selected based on successive "yes" or "no" answers to a series of questions regarding certain characteristics of the patient's condition. According to this model, as shown in Figure 3, F+F+K surgery should be indicated for the most severe motor and sensory spinal disorders, corresponding to A, B or C on the ASIA scale or, in the absence of neurological symptoms (E on the ASIA scale), or patients with types of tumors requiring radical surgery (Fig 3).

F+V surgery is performed only in cases of spinal neoplasms, allowing palliative surgery, and in the absence of manifestations of motor and sensory spinal disorders, while the vertebral lesion must occur without collapse of the vertebral body or be accompanied by a collapse of no more than 60%, or, in more severe lesions of the vertebra, spinal canal stenosis should correspond to the signs of stage I (with the spread of the neoplasm into the epidural space without deformation of the dural sac or deformation of the dural sac, but without signs of impact on the spinal cord) (Fig 3).

Signs for the appointment of F+F surgery are mild motor disfunction with preservation of normal sensitivity (D on the ASIA scale), or the absence of neurological symptoms in neoplasms that allow palliative intervention, or severe lesions of the vertebra with body collapse of more than 60% and the degree of epidural compression II or III stages (Fig 3).

Analysis of the results of the developed decision tree for cluster 1 showed that a type of surgical intervention was determined for 97 patients from 115 that relates to 84.3% of overall accuracy. Overall, the decision tree model showed the highest predictive accuracy for the F+V surgical intervention (100%), but was also predictively accurate for the F+F+K (80.0%) and F+F (83.72%) surgical interventions.

DISCUSSION

We developed mathematical model to select the extent of surgical management of the patients with malignant spine tumors by cluster analysis in retrospective study. This mathematical model allows to choose the most optimal from three types of surgical intervention with an accuracy of 84.3% for the treatment of patients with tumor lesions of the thoracic and lumbar vertebrae.

The progress in timely diagnosis, conservative treatment, and surgical management of oncologic pathology has allowed to increase the lifetime and life quality of patients. The decision on the advisability and treatment strategy of patients in this category is based on a multidisciplinary approach that considers not only the features of the spinal lesion but also the prognosis of the patient's survival, the presence of comorbidity, as well as a wide range of secondary factors that can significantly affect the non-final result of treatment.

Prognostic and diagnostic scales have been developed and improved, whereby it is possible to choose the best treatment tactics of patients to objectify decision-making over the past few decades.

Y. Tokuhashi et al. [20] and K. Tomita et al. [16] prognostic survival scales are known to evaluate the patient's survival outcomes, as well as the advisability and possibility of spinal surgical intervention. The Bauer, modified Bauer, Van der Linden, Rades and Katagiri scales are also used in clinical practice [21].

The development of the Spinal Instability Neoplastic Score has become a significant evolutionary step in the development of a systematic approach to the surgical management of spinal tumor lesions. This tool helps the surgeon to choose between standard diagnostic alternatives in oncology, specifically conservative, surgical management or palliative treatment, based on the location and extent of the pathological process of column [20].

The conceptual development of NOMS considers the features of neurologic presentation of spinal lesions, the specificity of the neoplastic process, the presence of metastases, as well as the assident general state of patient's health. The use of this framework facilitates a well-structured approach to the choice of treatment tactics based on consideration of most of the factors that can lead to poor treatment outcomes [22].

O. Barzilai et al. [23] has presented an algorithm of implementation of minimally invasive surgery in the treatment of metastatic tumor lesions in the thoracic and lumbar spine. The author concludes about algorithm effectiveness in terms of reducing post-surgical pain syndrome, quicker rehabilitation, and, consequently, improvement of life quality in cancer-stricken, based on the study of the results of this algorithm in 51 patients.

A common feature of most predictive algorithms and clinical decision-making paradigms is to build their structure and judge their effectiveness based on their clinical verification using the standard features that are specific to each tool. It is clear that this approach is quite effective, but in our opinion, it is too standardized. There is an opinion that further progress of medicine and oncology is particularly related to the personalization of diagnostic and therapeutic processes that allow to choose the most significant disease markers from the standard ones for a particular patient.

We have emphasized not the clinical statement in our work, but rather the mathematical background as the need for surgical management and a choice of specific surgical management, which is not coherent to other algorithms. The use of only mathematical methods in its development is a blindside of our study. In the years

ahead, there is a need for a prospective clinical verification of the obtained results, which will significantly improve the value of our findings.

CONCLUSIONS

We conducted a statistical analysis of prognostic criteria in patients with spinal neoplasms and developed a decision tree model using mathematical modeling to select the extent of surgical intervention. Our results showed that our mathematical model can accurately predict the most optimal surgical intervention with an accuracy of 84.3%. The model takes into account the neurological signs, type of surgery (radical or palliative), grade of vertebral lesion, and stage of epidural compression. Our mathematical model provides a quantitative approach to decision-making in clinical practice and may help to optimize surgical outcomes in these patients.

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ORIGINAL ARTICLE

EVALUATION OF D-DIMER LEVEL AS A BIOMARKER OF DISEASE SEVERITY AND MORTALITY IN PATIENTS WITH COVID-19

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ABSTRACT

The aim: To examine risk factors and evaluate the use of D-dimer as a biomarker of disease severity and mortality in patients with COVID-19.

Materials and methods: Data from a large NYU Langone Health system were analyzed to examine the prevalence of elevated D-dimer levels at first detection and the trend. A retrospective cohort study of 2,377 patients (NYU Langone Health) with severe COVID-19. Also we conducted a retrospective study based on the mortality database of 247 patients from COVID-19 at the Chernivtsi Regional Clinical Hospital.

Results: Patients with elevated baseline D-dimer were more likely to have critical illness than patients with normal D-dimer (43.9% vs. 18.5%). The frequency of adverse events increased with increasing D-dimer levels. Individuals with D-dimer >2000 ng/mL had the highest risk of critical illness (66.0%).

Conclusions: Thus, the level of D-dimer can be considered an important prognostic factor in COVID-19, as its level is elevated in the vast majority of patients with COVID-19 and correlates with a severe course and high mortality.

KEY WORDS: thrombosis, D-dimer, thromboembolism, coagulopathy, COVID-19

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INTRODUCTION

Numerous studies have shown that D-dimer is a valuable marker of coagulation activation and fibrinolysis. Normally, small amounts of D-dimer are detected in a healthy person due to the fact that approximately 2-3% of normally produced fibrinogen undergoes a continuous physiological cycle of fibrin formation and dissolution [1].

However, doctors in China, where the epidemic began, first reported that the level of D-dimer can be increased during COVID-19. A study of 191 patients with COVID-19 who were hospitalized in Wuhan in January 2020 at the start of the pandemic found that D-dimer levels were elevated in many of these patients, and the magnitude of the increase was greatest in those who died of the disease. A number of subsequent studies conducted around the world confirmed that D-dimer levels are elevated in those with severe COVID-19 and correlated with mortality rates [2].

In hospitalized patients with COVID-19, blood coagulation disorders, in particular, an increase in the level of D-dimer, fibrinogen, and an increase in prothrombin time are increasingly being detected [3].

Among adults admitted to the emergency department, the most common causes of elevated D-dimer levels are VTE (venous thromboembolism) and PE (pulmonary embolism). Previous studies in patients with community-acquired pneumonia (ACP) and chronic obstructive pulmonary disease (COPD) have shown that D-dimer levels are higher in severe cases and can be used as a prognostic biomarker [4].

However, the significance of D-dimer in patients with COVID-19 has not been fully investigated. We analyzed D-dimer levels in patient groups stratified by clinical severity, complications, and in-hospital death, and assessed the role of D-dimer as a biomarker of disease severity and clinical course.

THE AIM

The aim of the work is to examine risk factors and evaluate the use of D-dimer as a biomarker of disease severity and mortality in patients with COVID-19.

MATERIALS AND METHODS

We analyzed data from a large health care system at NYU Langone Health to examine the prevalence of elevated D-dimer levels at first detection and at baseline,

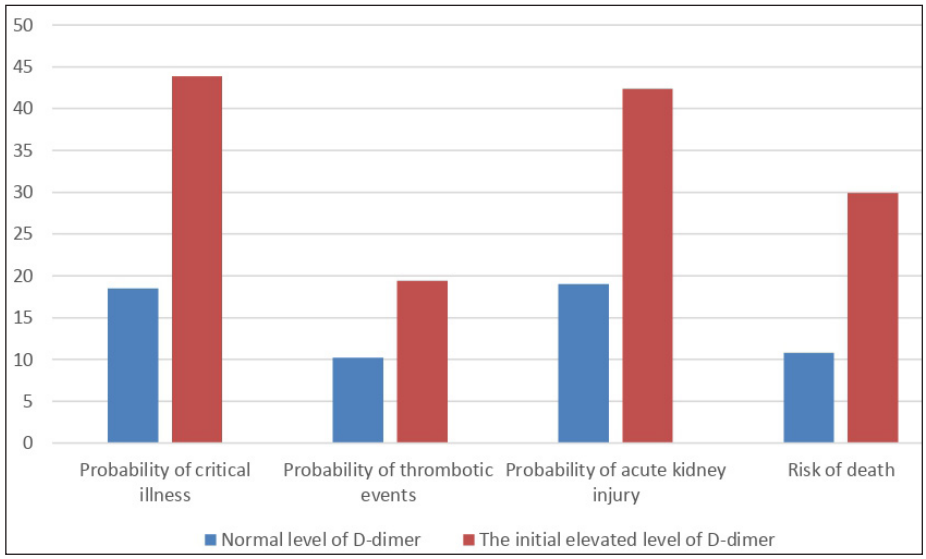

Fig. 1. The probability of developing serious conditions depending on the level of D-dimer

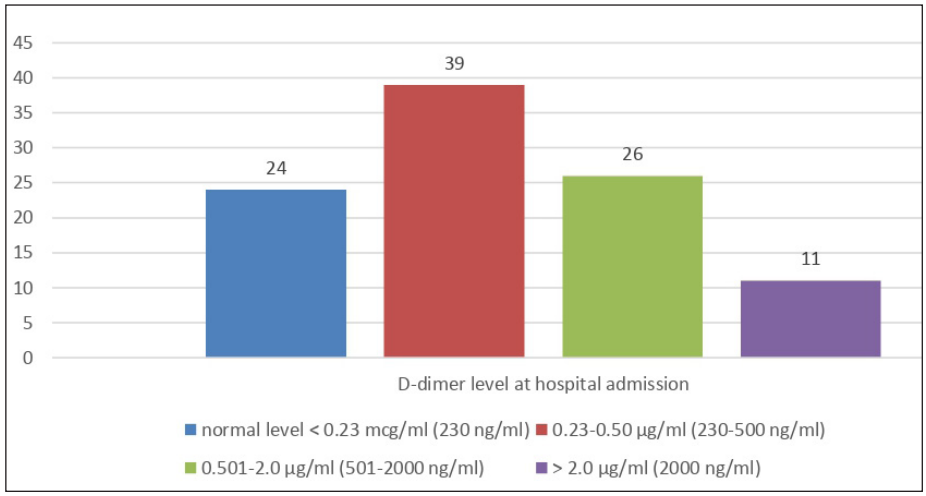

Fig. 2. The level of D-dimer at admission of patients to the hospital

and the association of the biomarker with thrombotic events, acute kidney injury, critical illness, and all-cause mortality. In a retrospective cohort study of 2,377 patients (NYU Langone Health) with severe COVID-19, 1,823 (76.0%) had elevated D-dimer above the laboratory upper limit of normal at hospital admission, 2,049 (86%) – an increase in the level of D-dimer during the period of hospitalization.

Also we conducted a retrospective study based on the mortality database of 247 patients from COVID-19 at the Chernivtsi Regional Clinical Hospital

RESULTS

In our analysis of clinical cases of patients with a diagnosis of COVID-19, elevation of D-dimer during hospitalization became a common phenomenon and was associated with both increased disease severity and in-hospital mortality. D-dimer can be synthesized only when the formation and degradation of cross-linked fibrin occurs, which is a global marker of activation of coagulation and fibrinolysis, and therefore reflects enhanced thrombotic activity [5]. Coagulation disorders such as hypercoagulability, thrombocytopenia, venous thrombosis, and disseminated intravascular coagulation (DIC) occur in approximately 60-70% of hospitalized patients. Autopsies showed that in nearly 58% of patients, the cause of death was pulmonary embolism or venous thrombosis, while CVD was reported in 70% of patients who died of COVID-19.

Numerous studies have also demonstrated an association between a trend in D-dimer levels and disease progression in COVID-19. Initial studies equated elevated D-dimer levels with CVD, but scientific studies have shown that coagulopathy associated with COVID-19 is a unique form. Innate immune response and endothelial damage contribute to coagulopathy associated with COVID-19. There is increasing evidence for an inflammatory component of coagulopathy associated with COVID-19, with one study even showing that an elevated white blood cell count during hospitalization is an independent predictor of VTE. Proposed contributing factors include endothelial damage, cytokine storm, complement activation, especially the alterna-

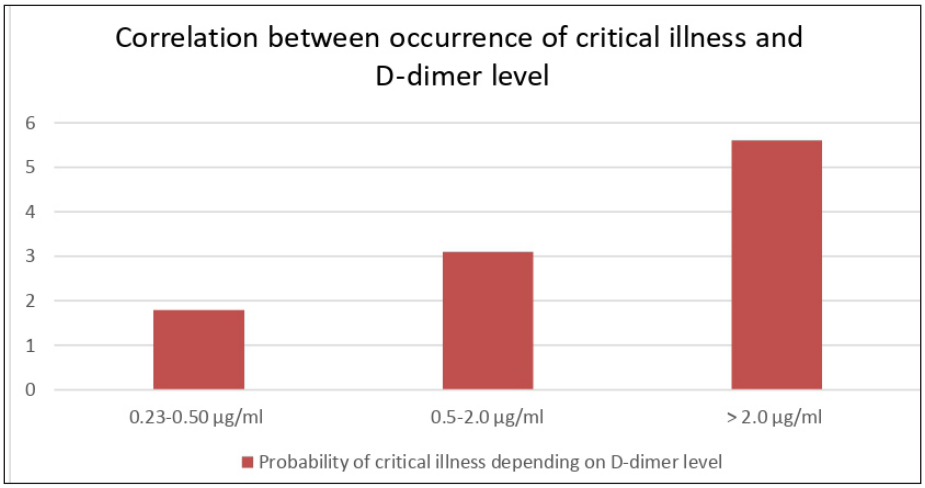

Fig. 3. The probability of a critical illness depending on the level of D-dimer

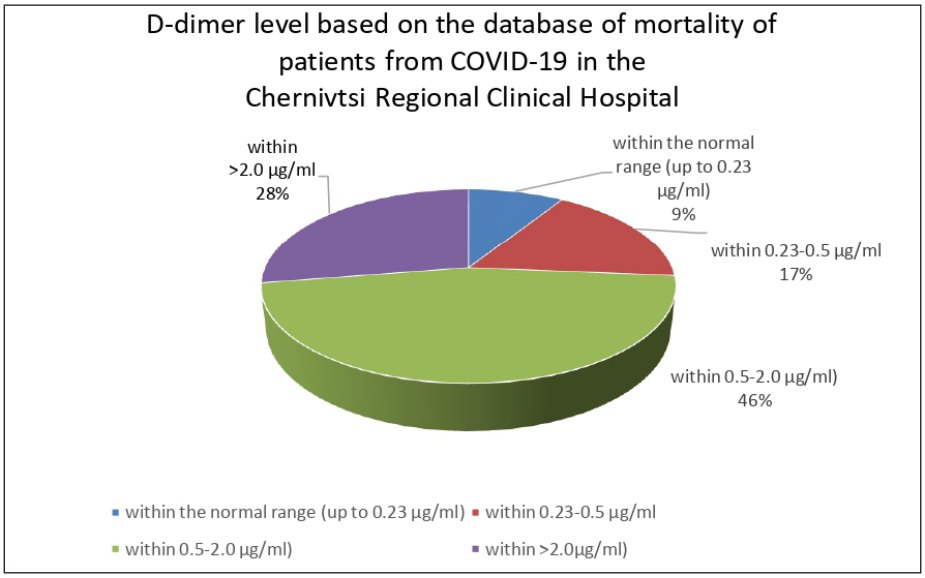

Fig. 4. D-dimer level in patients with COVID-19

tive pathway, which induces a hypercoagulable state and the formation of neutrophil extracellular traps [6].

It is currently known that acute lung injury in COVID-19 is associated with an increased frequency of thromboembolic events, which is a consequence of an imbalance between procoagulation factors and natural inhibitors of blood coagulation, cessation of fibrinolysis, endothelial damage, and the inflammatory process. It has been established that PE can be detected in 20-30% of patients with an acute course of COVID-19 [7].

D-dimer testing during hospitalization is routinely used in patients with COVID-19 in the healthcare system. The upper limit of normal for D-dimer analysis is 0.23 µg/ml (230 ng/ml). Currently, it is known that D-dimer can be not only a marker of hypercoagulation and prothrombotic state, but also participate in the pathogenesis of the disease. Fibrin breakdown products cause acute pulmonary dysfunction and have a direct procoagulant effect [8].

The D-dimer level is positively correlated with disease severity and inversely proportional to survival. Some case series report D-dimer values > 3 mg/L in 85% of patients who died after COVID-19, others D-dimer values > 1 mg/L in 81% of patients who died after COVID-19, and only 24% of surviving patients had a D-dimer level > 1 mg/l.

In early retrospective cohort studies of patients with COVID-19 in Wuhan, China, abnormalities in coagulation parameters including elevated D-dimer and prothrombin time were found. These coagulation changes were predictive of high mortality, with 15 of 22 (71.4%) failing International Society of Thrombosis and Haemostasis (ISTH) criteria for disseminated intravascular coagulation (DVT) compared with only 1 of 162 (0.6), which were confirmed in an early study [8]. By March 2020, the ISTH published an algorithm for the recognition and treatment of "Coagulopathy in COVID-19". Markers of coagulation disorders in COVID-19 currently being investigated include D-dimer levels, platelet count, von Willebrand factor (VWF), factor VIII, thromboelastography (TEG), lupus anticoagulant (LAC), antiphospholipid antibodies (APL), and fibrinogen [9].

In addition to abnormalities in laboratory markers of coagulation, COVID-19 infection has been associated with both venous and arterial thrombosis. Autopsy studies of patients with COVID-19 have shown both macro- and microvascular thrombosis. The SARS-Cov2 virus uses angiotensin-converting enzyme-2 (ACE-2) as its main receptor; this membrane protein is expressed in blood vessels, lungs, heart, kidney, and many other tissues. It has been hypothesized that the binding of SARS-CoV-2 to ACE-2 leads to a local and systemic inflammatory response, endothelial damage, and an imbalance of pro- and anticoagulant signals, leading to macro- and microvascular thrombosis [9].

During SARS-COV-2 infection, dysregulation of coagulation/anticoagulation cascades, increased viral replication and immune mechanism can be explained by an abnormal blood coagulation system, which includes both cellular and protein components. Considering the endothelial aggression and prothrombotic mechanisms caused by SARS-CoV-2, as well as the statistical results of the studies, we can say that the increased value of D-dimers can be prognostic for abnormal functional parameters of the liver and the severe course of the disease.

Presumably, elevated D-dimer represents a state of hyperfibrinolysis and increased inflammatory burden caused by SARS-COV-2 infection. In our logistic regression model to assess risk factors associated with mortality, systemic anticoagulation was not examined in detail. However, in a recent study that included 2,773 hospitalized patients with COVID-19, experts found that taking a therapeutic dose of anticoagulants was associated with a reduced risk of death, especially among patients who required mechanical ventilation.

The pathophysiology of coagulopathy associated with COVID-19 is an important factor in the appropriate treatment and monitoring of these complications. Experts emphasize the importance of diagnosis and treatment of coagulation disorders in COVID-19 to improve the outcomes of treatment of patients with COVID-19 with thromboembolic complications. Prolonged elevation of D-dimer may predict persistent ventilation-perfusion mismatch due to macro- and/or microthrombi and persistent inflammatory lung injury. Thus, elevated D-dimer levels may not only be a consequence of COVID-19, but also an associated comorbidity [10].

The results of patients at the time of hospitalization with an elevated D-dimer level were correlated with the following conditions: 45.0% of patients were in critical condition, 20.0% – with thrombosis, and 43.0% – with acute kidney injury. Individuals without elevated D-dimer at presentation were more likely to be discharged without developing severe conditions. Patients with elevated baseline D-dimer were more likely than patients with normal D-dimer to have critical illness (43.9% vs. 18.5%), any thrombotic event (19.4% vs. 10.2%); acute kidney injury (42.4% vs. 19.0%) and death (29.9% vs. 10.8%) (Figure 1).

It is currently known that the frequency of adverse events increased with increasing D-dimer levels. Individuals with D-dimer >2000 ng/mL had the highest risk of critical illness (66.0%), thrombosis (37.8%), acute kidney injury (58.3%), and death (47.0%). D-dimer was 387 (25-75th percentile), and 1823 (76.0%) patients had elevated D-dimer (>230 ng/ml). Median peak D-dimer was 767 (25th–75th percentile), and 2049 (86.0%) had elevated D-dimer >230 ng/mL at some point during hospitalization. Compared with patients with normal baseline D-dimer, patients with elevated baseline D-dimer were older (mean age 65) and had a lower body mass index (28.8). Among patients with elevated D-dimer, comorbidities were more common, including hypertension (63.5%), hyperlipidemia (44.2%), coronary heart disease (23.4%), and chronic kidney disease (23.0%) [8]. According to the results of studies, at admission, 1823 (76.0%) patients had elevated D-dimer (> 0.23 μg/ml (230 ng/ml)); 932 (39.0%) – in the range of 0.23-0.50 μg/ml (230-500 ng/ml), 628 (26.0%) – 0.501-2.0 μg/ml (501-2000 ng /ml), and 263 (11.0%) – > 2.0 μg/ml (2000 ng/ml) (Figure 2).

Of the total studied population, 899 (37.8%) patients had a critical illness (admitted to the intensive care unit), 620 (26.1%) needed mechanical ventilation, 410 (17.2%) had thrombotic events (DVT, PE, heart attack myocardial, ischemic stroke)) and 871 (36.8%) had acute kidney injury (AKI). Considering all these serious adverse clinical outcomes of COVID-19, patients with elevated D-dimer (> 0.23 μg/mL (230 ng/mL)) at the time of hospitalization were more likely to be affected than those with normal D-dimer (< 0.23 μg/ml (230 ng/ml)). For example, 43.9% of patients with elevated D-dimer at hospitalization compared with 18.5% of patients with normal D-dimer at hospitalization developed critical illness. Similarly, patients with elevated D-dimer on admission were more likely to require mechanical ventilation compared to patients with normal D-dimer levels (29.9% vs. 13.9%), and were more likely to suffer from APN (42.4% vs. 19.0%) and from thrombosis (19.4% versus 10.2). %).

It was found that the magnitude of the D-dimer increase upon admission to the hospital is independently associated with the risk of serious clinical consequences. After controlling for age, sex, ethnicity, defined list of comorbidities, and prescribed treatment, critical illness was 1.8 times higher if D-dimer was in the range of 0.23-0.50 μg/ml (230- 500 ng/ml) than normal (< 0.23

μg/ml (230 ng/ml)), 3.1 times more likely if D-dimer was in the range of 0.5-2.0 μg/ml (500-2000 ng/ml) and 5.6 times more likely if D-dimer at admission was > 2.0 μg/ml (2000 ng/ml) (Figure 3).

In addition, we conducted a retrospective study based on the database of mortality of patients from COVID-19 in the Chernivtsi Regional Clinical Hospital. It was established that the elevated level of D-dimer was noted in 225 out of 247 patients with coronavirus disease, which is 91.0%. It was found that in all patients the level of D-dimer varied in the range: within the normal range (< 0.23 μg/ml (230 ng/ml)) – in 22 people (8.9%); 0.23-0.50 μg/ml (230-500 ng/ml) – in 43 people (17.4%); in the range of 0.5-2.0 μg/ml (500-2000 ng/ml) – in 114 people (46.2%); and more than 2.0 μg/ml (2000 ng/ml) in 68 people (27.5%) (Figure 4).

Thus, the results of D-dimer studies should be used as a biomarker to assess clinical outcomes in patients with COVID-19. It is worth noting that the obtained data indicate a connection between the levels of D-dimer and the severity of the disease and mortality, which is also confirmed by the data of other scientists.

DISCUSSION

When assessing the trajectory of D-dimer elevation during COVID-19, it was found that D-dimer levels continued to rise after hospitalization, reaching a maximum level around day 5 before gradually plateauing at a level below the peak but greater than, than the level at hospitalization. Median D-dimer for the entire study population at admission was 0.387 mcg/mL (387 ng/mL) (25th-75th percentile range: 0.237-0.713 mcg/mL (237-713 ng/mL)) compared with a median peak of 0.767 μg/mL (767 ng/mL) (25th-75th percentile: 0.328-3.372 μg/mL (328-3372 ng/mL)) after approximately 5 days. The results of the study demonstrate that the magnitude of the peak D-dimer level, as well as the magnitude of the D-dimer level at hospitalization, correlates with the risk of an adverse clinical course. It was found that of the total study population, 301 (12.7%) patients had a peak D-dimer level > 10.0 μg/ml (10,000 ng/ml). This group of patients with the highest peak D-dimer levels had the highest incidence of serious clinical outcomes during hospitalization: 86.0% had critical illness requiring admission to the intensive care unit; 71.0% required mechanical ventilation; 81.0% – had GPN; and 39.0% had a thrombotic event.

In addition to the association between D-dimer levels and adverse clinical outcomes, we also examined the relationship between D-dimer levels at admission and the final outcome for patients with COVID-19: death or recovery.

The data we received also confirm the research of other scientists. A retrospective study by W.J. Guan et al. (2020) (n=1099) established a relationship between the severity of the course of COVID-19 and a high level of D-dimer. The level of D-dimer ≥0.5 mg/l was noted in 46.4% of patients, and 60% of them developed severe manifestations of the disease [11]. Another retrospective study (n=183) found that the level of D-dimer in patients with a severe course of the disease is almost 3.5 times higher than in patients with a mild or moderate course [12]. In addition, it is noted that D-dimer is a marker of fibrin deposition in the lungs. The experience of Spanish doctors established that patients with high levels of D-dimer and C-reactive protein more often required hospitalization and transfer to artificial lung ventilation [13].

CONCLUSIONS

D-dimer level is an important tool for early triage and monitoring of patients with COVID-19, a prognostic marker of severe course and death due to SARS-CoV-2 coronavirus disease. D-dimer levels were independently associated with a higher risk of critical illness, thrombosis, acute kidney injury, and all-cause mortality among patients with COVID-19.

Thus, the level of D-dimer can be considered an important prognostic factor in COVID-19, which correlates with a severe course and high mortality.

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ORIGINAL ARTICLE

THE EFFECT OF *RUTA GRAVEOLENS L.* ETHANOLIC EXTRACTS ON SKIN ISOLATES OF *STAPHYLOCOCCI* AND *PROPIONIBACTERIUM ACNES*

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ABSTRACT

The aim: To define antimicrobial properties of 50%, 70% and 90% ethanolic extracts of *Ruta graveolens L.* against macrolide resistant (MLS-resistant) skin isolates of *staphylococci* and *Propionibacterium acnes*, and to determine MIC and MBC of investigated extracts.

Materials and methods: Extracts were prepared by the method of maceration. Bacterial cultures were identified by biochemical microtests. Identification of MLS-resistance type was performed by using disc-diffusion method. The MIC and MBC were determined by serial two-fold dilution of ethanolic extracts of *Ruta graveolens L.* in MHB and HBB for *staphylococci* and *P. acnes*, respectively. Bacterial growth in each well was assayed by absorption at 495 nm, using a spectrophotometer SynergyTMHTX S1LFTA (BioTek Instruments, Inc., USA).

Results: All ethanolic extracts of garden ruta were active against all *staphylococci* and *P. acnes* skin isolates and showed exclusively bactericidal activity (MBC/MIC ratios ranged from 1 to 2) against all investigated strains. 90% extract of *Ruta graveolens L.* showed better results than 50% and 70% extracts – average MIC and MBC concentations for *P. acnes* strains were 1.38±0.66 mg/mL and for staphylococcal strains average MIC was 2.1±1.16 mg/mL and MBC – 2.86±1.2 mg/mL.

Conclusions: 50%, 70% and 90% ethanolic garden ruta extracts showed moderate antibacterial activity against main skin pathogens, responsible for acnes vulgaris development – *S. epidermidis*, *S. aureus* and *P. acnes*. No difference in susceptibility between resistance and sensitive strains of *staphylococci* and *P. acnes* indicate that acquired MLS-resistance of investigated skin isolates does not affect on the level of their sensitivity to ruta extracts.

KEY WORDS: antibacterial activity, plant extracts, MLS-resistance

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INTRODUCTION

The homeostasis of cutaneous microbiome is important to its function as a barrier against infectious agents and colonization of skin surface by pathogens. Three most observed genera of skin ecosystem – *Corynebacteria, Propionibacteria* and *Staphylococci*. Disturbance of skin microbiome can lead to various pathological conditions and inflammatory skin diseases, such as acnes vulgaris [1-3].

Nowadays, increasing in antibiotic resistance among microorganisms is a global public health concern. That usually happens because of indiscriminate use of antimicrobials, careless prescription or selling of antibiotics without a prescription by doctors (which is appear in a lot of countries in the world) [4, 5]. Furthermore, increasing of antimicrobial resistance among skin pathogens can be as a result of long-term administration and/or low-concentration exposure of oral antimicrobials (usually macrolides) [6]. However, using of topical treatments in therapy of skin disorders less likely cause antibiotic resistance [7].

Such increasing in number of multidrug-resistant strains of microorganisms and development of strains with low susceptibility to antimicrobials drives scientists from different countries to discover new antimicrobial agents and overcome antibiotic resistance. Such agents may include plant extracts, which have substantive antimicrobial potential [8], have less side effects than antibiotics and are cheaper in manufacturing.

Among variety of plants with medicinal interest, there is *Ruta graveolens* which belongs to family Rutaceae and contain a huge amount of biologically active compounds such as essential oils, alkaloids, flavonoids, coumarins, tannins, volatile oils, sterols and triterpenes [9]. Moreover, pharmacological trials indicate a variety of activities, such as antipyretic, anti-inflammatory, antioxidant, antiulcer, wound healing, anthelmintic, antiseptic, antifungal and antimicrobial properties [10-12]. Furthermore, some studies reported antitumor activity of *Ruta graveolens L.* [13, 14].

Table I. Strains characteristics

Strains	Associated resistance	Phenotype of MLS-resistance	MIC ERY (μg/mL)
Staphylococci:			
S. aureus №1	MR, TR	resistant	2000
S. aureus №2	-	susceptible	500
S. epidermidis №1	MR	resistant	8000
S. epidermidis №2	MR	resistant	4000
S. epidermidis №3	-	resistant	2000
S. epidermidis №4	MR, TR, QR	resistant	500
S. epidermidis №5	TR	constitutive	500
S. epidermidis №6	-	constitutive	500
S. epidermidis №7	-	inducible	500
S. epidermidis №8	QR	susceptible	16
S. haemolyticus №1	MR, QR	resistant	4000
Propionibacterium acnes:			
P.acnes №1	MR, QR	resistant	4000
P.acnes №2	MR, TR, QR	resistant	2000

Notes: MR – methicillin resistant; TR – tetracycline resistant; Q – quinolones resistant.

In our previous study [16] we established that 50%, 70% and 90% ethanolic extracts of *Ruta graveolens* L. herb are more active, than 40% and 96% extracts, and staphylococci are the most susceptible microorganisms to garden ruta extracts.

THE AIM

To define antimicrobial properties of 50%, 70% and 90% ethanolic extracts of *Ruta graveolens* L. against main causative agents of skin diseases – skin isolates of staphylococci and *Propionibacterium acnes*, especially emphasized macrolide resistant (MLS-resistant) isolates, and to determine MIC and MBC of investigated extracts.

MATERIALS AND METHODS

PLANT EXTRACTS

In our investigation we used 3 water-ethanolic extracts of garden ruta (extractants – 50%, 70% and 90% ethanol) herb. Extracts were prepared by the method of maceration (according to the recommendations of the State Pharmacopoeia of Ukraine). This maceration is the re-extraction of the original plant material in separate portions of fresh extractant. The total amount of extractant was divided into 3 parts and successively infused raw materials with the first part of extractant, then with second and third one, each time draining the extract. For complete extraction of biologically active substances, the infusion of raw materials was carried out with constant shaking. The infusion time lasted for 24 hours with the first portion of the extractant and for 2 hours with each subsequent portion of extractant. Such extraction allows deplete the raw material completely in shorter time, because of constantly maintained high difference in concentration of raw material and the extractant [16]. The aliquots 1.0 mL of prepared extracts have been dried exhaustively at room temperature to determine the weight of dry residue of extracted components (Table II).

BACTERIAL STRAINS

Investigation of antimicrobial activity of garden ruta extracts were performed on 13 strains of skin isolates. Bacterial cultures were identified by biochemical microtests «STAPHYtest 16» (Lachema, Czech Republic) as 2 stains of *S. aureus*, 8 strains of *S. epidermidis* and 1 *S. haemolyticus* strain, and by «ANAEROtest 23» (Lachema, Czech Republic) as 2 strains of *P. acnes*.

Identification of MLS-resistance type was performed by using disc-diffusion method, by recommendation of EUCAST (The European Committee on Antimicrobial Susceptibility Testing). Susceptibility to 6 antibiotics was examined: erythromycin (E, 15 μg/disc), clarythromycin (CLR, 15 μg/disc), roxithromycin (RO, 30 μg/disc), spiramycin (SR, 30 μg/disc), lincomycin (L, 15 μg/disc) and clindamycin (CD, 2 μg/disc). In addition, for the detection of MLS-resistant phenotypes (inducible or constitutive), the double disc-diffusion test (D test)

Table II. Minimum inhibitory and minimum bactericidal concentrations of *Ruta graveolens L.* ethanolic extracts on skin isolates of *staphylococci* and *Propionibacterium acnes*.

Strains of *staphylococci*	**MLS-resistance phenotype**	**Ethanolic extracts of *Ruta graveolens L.***								
		50%			**70%**			**90%**		
Weight of dry residue		**109 mg/mL**			**110 mg/mL**			**74 mg/mL**		
		MIC	**MBC**	**MBC/ MIC**	**MIC**	**MBC**	**MBC/ MIC**	**MIC**	**MBC**	**MBC/ MIC**
1. *S. aureus* №1	R	1:40* 2.73**	1:40 2.73	1	1:40 2.75	1:40 2.75	1	1:40 1.85	1:40 1.85	1
2. *S. aureus* №2	S	1:40 2.73	1:20 5.45	2	1:20 5.5	1:20 5.5	1	1:160 0.46	1:80 0.92	2
3. *S. epidermidis* №2	R	1:40 2.73	1:40 2.73	1	1:40 2.75	1:20 5.5	2	1:20 3.7	1:20 3.7	1
4. *S. epidermidis* №1	R	1:20 5.45	1:20 5.45	1	1:40 2.75	1:20 5.5	2	1:40 1.85	1:20 3.7	2
5. *S. haemolyticus* №1	R	1:40 2.73	1:40 2.73	1	1:40 2.75	1:40 2.75	1	1:20 3.7	1:20 3.7	1
6. *S. epidermidis* №3	R	1:80 1.36	1:40 2.73	2	1:80 1.38	1:40 2.75	2	1:160 0.46	1:80 0.92	2
7. *S. epidermidis* №4	R	1:40 2.73	1:40 2.73	1	1:40 2.75	1:40 2.75	1	1:40 1.85	1:20 3.7	2
8. *S. epidermidis* №6	C	1:40 2.73	1:40 2.73	1	1:40 2.75	1:40 2.75	1	1:40 1.85	1:20 3.7	2
9. *S. epidermidis* №5	C	1:40 2.73	1:40 2.73	1	1:40 2.75	1:40 2.75	1	1:40 1.85	1:20 3.7	2
10. *S. epidermidis* №7	I	1:40 2.73	1:40 2.73	1	1:80 1.38	1:40 2.75	2	1:20 3.7	1:20 3.7	1
11. *S. epidermidis* №8	S	1:40 2.73	1:40 2.73	1	1:40 2.75	1:40 2.75	1	1:40 1.85	1:40 1.85	1
Avarage		2.85± 0.95	3.22± 1.1		2.75± 1.06	3.5± 1.28		2.1± 1.16	2.86± 1.2	
GeoMean		2.73	3.09		2.58	3.32		1.74	2.53	
12. *P.acnes* №2	R	1:80 1.36	1:40 2.73	2	1:80 1.38	1:80 1.38	1	1:80 0.92	1:80 0.92	1
13. *P.acnes* №1	R	1:40 2.73	1:40 2.73	1	1:80 1.38	1:40 2.75	2	1:40 1.85	1:40 1.85	1
Avarage		2.05± 0.97	2.73		1.38	2.06± 0.97		1.38± 0.66	1.38± 0.66	
GeoMean		1.93	2.73		1.38	1.95		1.3	1.3	

Notes: R – resistant, S – susceptible, C – constitutive, I – inducible;
*Extract dilutions
**MIC and MBC expressed in mg/mL

was applied, by placing erythromycin and clindamycin discs 12-20 mm apart (edge to edge). D-shaped zone around disc with clindamycin, induced by erythromycin to be resistant and reported as inducible resistance [17]. Strains characteristic are presented in Table I.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC), MINIMUM BACTERICIDAL CONCENTRATION (MBC) AND LIQUID TIME – KILL CURVE METHOD

Bacterial suspensions of staphylococci (10^6 CFU/mL) were inoculated into Mueller-Hinton liquid broth (MHB) and Heart-brain broth (HBB) was used for *P.acnes* (10^7 CFU/mL), dispensed at 100 µl/well in a 96-well microtiter plate. The MIC and MBC were determined by serial two-fold dilution of ethanolic extracts of *Ruta graveolens L.* in MHB and HBB for *staphylococci* and *P. acnes*,

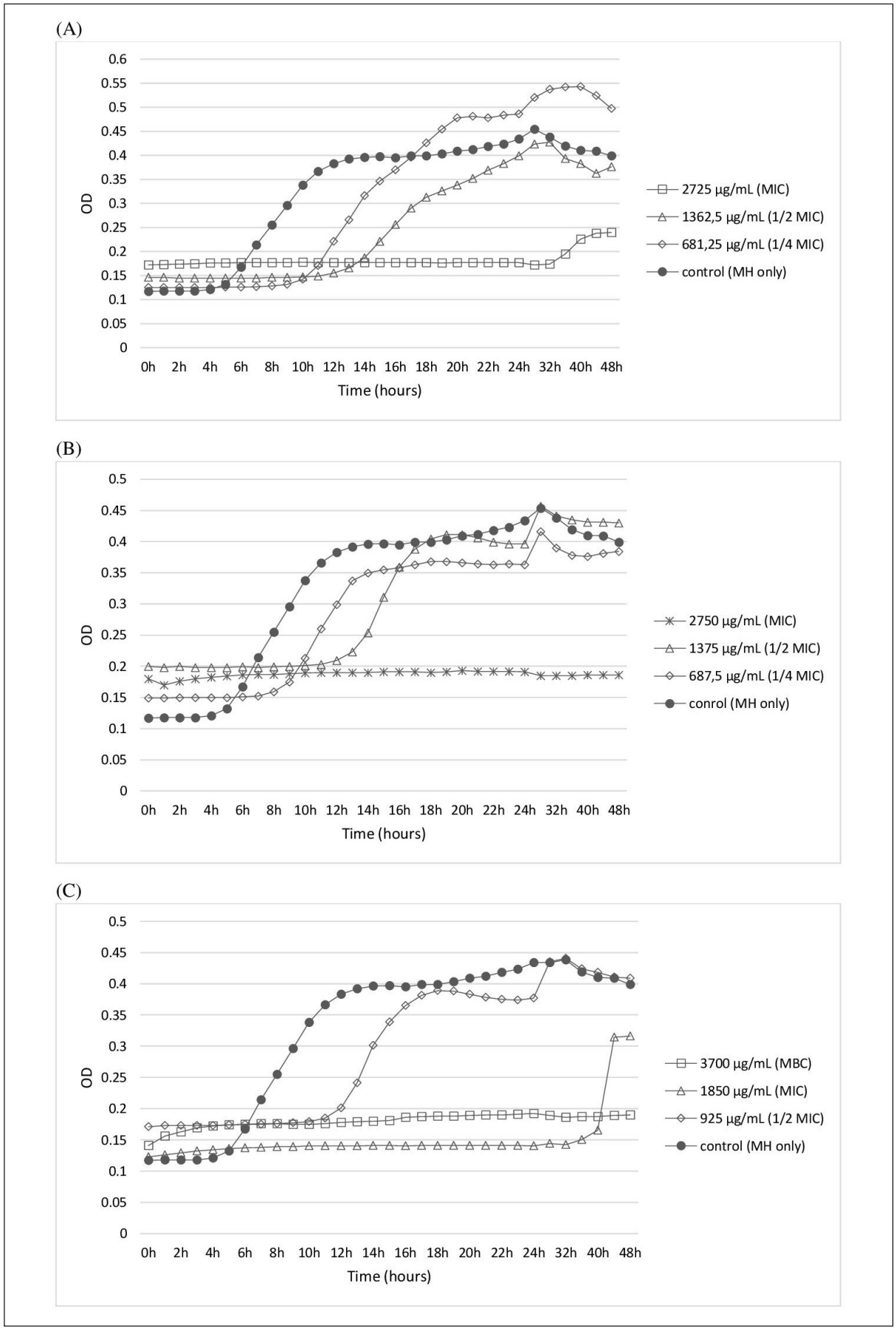

Fig. 1. Time-kill curves of 50% (A), 70% (B) and 90% (C) *Ruta graveolens L.* extracts on multiple resistant strain *S. epidermidis* №4

respectively. After incubation at 37°C for 24-48 hours for staphylococci and 48-72 hours for *P. acnes*, bacterial growth (increasing of OD) in each well was assayed by absorption at 495 nm, using a spectrophotometer Synergy™HTX S1LFTA (BioTek Instruments, Inc., USA). Culturing of *P. acnes* strains was carried out under anaerobic condition provided by Gas generation pouch system (GasPak™ EZ, Bacton, Dickinson and Co, USA).

MIC for staphylococci was determined after 24 hours of incubation, as the lowest concentration of extract that inhibits visible bacterial growth, compared to control wells (microbial culture only). If during 48 hours of incubation there was no increase in OD by more than 10%, then this concentration of extract was considered as MBC. MIC and MBC for *P. acnes* was determined after 48 and 72 hours, respectively.

Bacteriostatic activity has been defined as a ratio of MBC to MIC of > 4, while a bactericidal action has the ratio of MBC to MIC is ≤ 4 [18].

To further confirm the bactericidal activity of extracts under study, time-kill curves was performed. Monitoring of bacterial growth kinetic was performed by OD measuring every 1 hour for a period of 48 hours. The growth curves were built using the Gen 5 Software (Figure 1).

STATISTICAL ANALYSIS

All experiments were performed in triplicate and results were expressed as the mean ± standard deviation (SD). Statistical analyses were performed by ANOVA, GEN5 and Microsoft Office Excel 2011.

RESULTS

All ethanolic extracts of garden ruta showed antimicrobial activity against all *staphylococci* and *P. acnes* skin isolates.

MICs of 50% ethanolic extract against most tested strains were in a dilution 1:40 (activity ranged between dilution of extract from 1:20 to 1:80), whereas bactericidal activity of this extract was in dilution 1:40 against 11 investigated strains and 1:20 against only 2 strains. Ruta extract on 70% of ethanol showed the same activity as 50% extract – bacteriostatic and bactericidal activity were detected in dilution 1:40 for 8 *staphylococcal* strains, whereas *P. acnes* were more susceptible for this extract (MIC were 1:80 for both strains). More diverse MIC and MBC were showed by 90% ethanolic ruta extract: MIC values were 1:20 against 2 tested strains, 1:40 – against 7 strains, 1:80 only for one *P. acnes* and 1:160 against 2 staphylococcal strains. MBC values of 90% ethanolic extract were in dilution 1:20 against 7 strains of staphylococci, 1:40 – against 2 *S. epidermidis* strains and 1 *P. acnes* strain and in dilution 1:80 – against 3 tested strains (2 strains of *staphylococci* and 1 strain of *P. acnes*).

Active concentrations of extracted components were calculated as weight of dry residues (after evaporation of 1.000 mL of extract at room temperature) divided into dilution and were presented in mg/mL (Table II).

The MIC values of 50%, 70% and 90% *Ruta graveolens L.* ethanolic extracts ranged from 1.36 to 5.45 mg/mL, 1.38 to 5.5 mg/mL and 0.46 to 3.7 mg/mL, respectively. Minimum bactericidal concentrations ranged from 2.73 to 5.45 mg/mL, 1.28 to 5.5 mg/mL and 0.92 to 3.7 mg/mL for 50%, 70% and 90% ethanolic extracts, respectively. Also, strains of *P. acnes* were more susceptible to ruta herb extracts (MIC of 50% – 2.05±0.97 mg/mL; of 70% – 1.38 mg/mL and of 90% – 1.38±0.66 mg/mL) than strains of staphylococci (MICs of 50%, 70%, 90% were 2.85±0.95 mg/mL, 2.75±1.06 mg/mL and 2.1±1.16 mg/mL, respectively). 90% extract of *Ruta graveolens* L. showed better results than 50% and 70% extracts – average MIC and MBC concentations for *P. acnes* strains were 1.38±0.66 mg/mL and for *staphylococcal* strains average MIC was 2.1±1.16 mg/mL and MBC – 2.86±1.2 mg/mL.

Additionally, the GeoMean value for MIC of 90% ruta extract were lower (1.74 mg/mL for *staphylococci* and 1.3 mg/mL for *P. acnes* strains) than for other investigated extracts (for 50% extract GeoMean was 2.73 mg/mL for *staphylococci* and 1.93 mg/mL for *P. acnes* strains; for 70% ruta extract GeoMean was 2.58 mg/mL for staphylococci strains and 1.95 mg/mL for *P. acnes* strains). Average MIC and MBC were almost the same for 50% and 70% ruta extracts, and indicate that there is almost no difference between this two extractants in a preparing of ethanolic extracts from *Ruta graveolens L.*

Also, all ruta extracts showed exclusively bactericidal activity (MBC/MIC ratios ranged from 1 to 2) against all investigated strains (Table II).

There was no difference in susceptibility between resistance and sensitive strains of *staphylococci* and *P.acnes*. So, acquired MLS-resistance of investigated strains of skin isolates does not affect on the level of their sensitivity to ruta extracts.

Analysis of *staphylococci* growth curves (Fig. 1) demonstrates substantial prolongation of initial lag-phase in the media supplemented with ruta extracts (8-10 hours for ¼ MIC of extracts, 12-13 hours for ½ MIC of extracts) comparing with the pure MH broth (4 hours). Duration of exponential log-phase was mostly unchanged. But testing of 50% ruta extract in ½ MIC and ¼ MIC revealed two-phase character of culture exponential growth. The rapid exponential OD increase for 4 hours was followed by slowing down period of OD increase for the next 5-7 hours before achieving plateau.

DISCUSSION

Various plants are used worldwide in a traditional medicine for treatment of bacterial infections. Although most of them have been tested *in vitro*, bur more controlled clinical trials are needed to confirm effectiveness of such herbal drugs [19]. The antimicrobial properties of plants are associated with the presence of various biologically active substances, such as flavonoids, alkaloids, tannins, terpenoids and essential oils [20]. Also, there are many studies on the possibility of using plants in the treatment of skin diseases, including acne [21-23].

Acnes vulgaris is a chronic inflammatory disorder of skin (pilosebaceous follicles) that affects usually adolescents and young people. Pathogenesis of acnes includes increasing in sebum production, ductal hypercornification, development of inflammation and colonization of the duct by *P. acnes* [24]. Other microorganisms which are often isolated from patients with *acne vulgaris* include *Staphylococcus epidermidis, Malasezia furfur and Staphylococcus aureus*, which is the most common nosocomial pathogen with high level of antibiotic resistance [24, 25]. That is why in our research we used *staphylococci* and *Propionibacterium* acnes skin isolates as tested strains. Besides, *staphylococci* are considered as gold standard in the study of antibacterial action of antibiotics and other antimicrobial agents, especially plant extracts [26].

Ruta graveolens L. is known to have long been used in folk medicine, as a plant with numerous biological activities, including antimicrobial [10-14]. Such a variety of actions corresponds to the rich chemical compositions of the plant [9]. Secondary metabolites of garden ruta herb like tannins have natural astringent properties and can be used topically in a treatment of acnes [27]. Essential oils of *Ruta* species are reported like substances with antimicrobial potential [10]. Also, numerous studies demonstrated that *Ruta graveolens* L. possess antioxidative action, which is one of important aspect in acne treatment [11].

In our study we evaluated antibacterial action of *Ruta graveolens L.* organic extracts against main causative agents of acne vulgaris (*P. acnes, S. epidermidis* and *S. aureus* skin isolates). That is the first report about antimicrobial potential of ruta herb in a treatment of acne.

CONCLUSIONS

Multiple antibiotic resistance among microorganisms is a continuing problem in whole world. The search for alternatives with antimicrobial activity remains relevant. The results of our study indicate the antimicrobial potential of *Ruta graveolens L.* biologically active substances.

We evaluated antibacterial activity of 50%, 70% and 90% ethanolic garden ruta extracts against such skin pathogens as *S. epidermidis, S. aureus and P. acnes*. Becides, this is first report about susceptibility of main causative agents of acnes vulgaris to *Ruta graveolens L.* extracts.

Although the active concentrations of investigated extracts indicate moderate activity, but we can suspect possibility of synergistic interactions with antibiotics that can help to overcome antimicrobial resistance.

Further research is needed to identify active compounds and study other biological activities of *Ruta graveolens L.* extracts and their toxicity.

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

INFLUENCE OF BAD HABITS ON THE DEVELOPMENT OF ACQUIRED DEFORMATIONS IN THE MAXILLOFACIAL AREA

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ABSTRACT

The aim: Identifying the relationship between the presence of oral habit and acquired maxillomandibular anomalies, influence of oral habits on the skeleton and muscular system formation in children.

Materials and methods: We conducted clinical, radiological methods of examination of 60 patients aged 9-12 with acquired maxillomandibular anomalies, 15 persons aged 9-12 years without maxillomandibular anomalies and acquired deformities (norm group) and 15 persons aged 9-12 years with hereditary syndromes, which are combined with bone deformities in the maxillofacial area (comparison group).

Results: Clinical examination showed that oral habits were manifested in 98.3% of patients. The results of clinical and radiological examination, analysis of cephalometric parameters and data on the thickness of the masticatory muscles on symmetrical areas of the face confirm the relationship between chronic oral habits and formation of acquired maxillomandibular anomalies; confirm the presence of acquired rather than congenital deformity of the facial skeleton, which is associated with changes in the thickness of the masticatory muscles on the part of the deformation та compensatory muscle hypertrophy on the opposite side.

Conclusions: The oral habit should be considered as one of the triggers in the development of acquired deformities of the maxillofacial area.

KEY WORDS: cephalometric analysis, acquired deformities of the maxillofacial area, oral habits

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INTRODUCTION

According to the data of a whole galaxy of scientists, the prevalence of dento-jaw anomalies ranges from 35.0% to 75.0% among all pathologies of the maxillomandibular system [1-5]. Scientists emphasize various etiopathogenetic chains in the development of anomalies and deformations. However, often the cause of acquired abnormalities are oralhabits of patients. Today, the problem of oral habits in children with maxillomandibular anomalies and acquired deformities is relevant, as they are progressing faster and more intensively among young people who are in distance learning. Children use oral bad habits very often supporting the head with hands, causing chronic injury in this area, sitting in front of a monitor with his mouth open, despite a positive breath test (presence of nasal breathing), keeping fingers in the mouth, pencils, biting nails, pencils or pens. Isolated studies indicate that the systematic use of oral habits in causes changes in the facial skeleton and maxillofacial area [3, 6-9]. We suggest that oral habits, especially those associated with prolonged mechanical exposure to the facial skeleton and jaw bones, are the trigger for deformation in cellular mechanotransduction of the functional matrix of the skeletal system and is the phenotypic expression of the human body. Combining discoveries from cellular mechanotransduction with the theory of biological networks, it forces scientists to reconsider the existence of a functional matrix developed by E. Moss and the influence of genotypic expression on bone formation. Anvils indicate the presence of different types of intracellular mechanotransduction processes. They translate the information content of the stimulus of the periosteal functional matrix into the cell signal of the skeletal unit (bone). Scientists emphasize the correlation between the intensity and duration of endogenous electric fields created by skeletal muscle activity and those to which bone cells respond best. Phenotypic expression triggers a chain of macromolecular levers that connect the extracellular matrix to the bone cell genome, suggesting a different way of epigenetic regulation of the bone cell genome. Intercellular slit connections allow bone cells to transmit and then process information of the periosteal functional matrix after its initial intracellular mechanotransduction [10, 11, 5]. Physical forces play an important role in modulating cell function and shaping tissue structure. Mechanotransduction, the process by which cells convert physically induced signals into bio-

chemical reactions, is critical for mediating adaptation to mechanical stress in connective tissues [12, 6, 11].

Scientists emphasize that slit connections, like electrical synapses, underlie the organization of bone tissue as a connected cellular network and the fact that all bone adaptation processes are multicellular. The bone is "tuned" to the exact frequency of skeletal muscle activity. The inclusion of concepts and databases related to intracellular and intercellular mechanisms and processes of bone cell mechanotransduction and organization of bone as a biologically related cellular network allows us to reconsider the functional matrix hypothesis, which offers an explanatory chain extending from the epigenetic event of muscle contraction hierarchically down to the regulation of the bone cell genome [11, 13]. We suggest that intercellular mechanotransduction is a critical component in achieving coordinated remodeling responses to the application of force in connective tissues, which requires further study and research.

Without modern diagnostic methods, it is not always a simple task to establish what kind of abnormality the patient has. 2D diagnostics (orthopantomogram, 2D lateral and direct cephalography) allows studying a number of angular and linear parameters. Often, the orthodontist needs additional X-ray examinations, which is associated with two-dimensional flattening, variable magnification of various anatomical structures of the facial skull and limitation due to total overlap. We can replace 2D diagnostics with three-dimensional reconstruction using cone-beam computed tomography. The patient will receive a lower dose of radiation than with multispiral computed tomography, but more than with 2D diagnostics. 3D cephalometry allows you to visualize the bone structure of the maxillofacial area and muscles, study and evaluate the anatomical structures of the facial skull in thin sections in all three planes, study the base of the skull to understand the patient's congenital or acquired deformity, help the orthodontist avoid diagnostic errors

THE AIM

Identifying the relationship between the presence of oral habit and acquired maxillomandibular anomalies, influence of oral habits on the skeleton and muscular system formation in children.

MATERIALS AND METHODS

We conducted clinical, radiological methods of examination of 60 patients aged 9-12 with acquired maxillomandibular anomalies, 15 persons aged 9-12 years without maxillomandibular anomalies and acquired deformities (norm group) and 15 persons aged 9-12 years with hereditary syndromes, which are combined with bone deformities in the maxillofacial area (comparison group).

We conducted a secret survey to identify a oral habit in patients. We conducted a secret survey to identify a oral habit. We conducted a photo protocol and processed the results using AutoCAD 2007. Muscle hypertonia musculus oblicularis oris was determined. The presence of such pathology indicates problems with swallowing (excessive muscle tension), speech and rest. We performed a respiratory test to detect nasal breathing in pat ients, necessarily during the external oral examination.

We studied and analyzed the data of computed tomograms of 60 patients with acquired maxillomandibular anomalies, deformities, 15 tomograms of persons of the norm group. X-ray methods included examination of the patient on a spiral computed tomography scan TOSHIBA Aquilion PRIME 160-slices MODEL TSX-302A / 1C. The scan was performed according to a specially developed protocol. During the scan, the position of the jaws in the bite and the head remains stable in order to reduce the risk of artifacts. The reconstruction algorithm at the time of the study was set as "bone" or "high resolution". The matrix extension was 512x512. The scan range included the facial and cerebral skulls. The thickness of the slice during the scan was 3-5 mm, the step in the reconstruction of the slice was 1 mm. All sections matched the anatomical area, had the same proportions and sizes and were scanned at the same table height. The scan was performed in one direction. After the study, archival data were stored in Dikom format.

The main method of examination is stereotopometric analysis (three-dimensional cephalometry), which studied the ratio of the structures of the facial head relative to three mutually perpendicular planes. Three-dimensional cephalometric analysis was performed on computer reconstructions in SimPlant Pro 11.04 software. SurgiCase (Materialize) was used according to the developed modified method of cephalometric and stereotopometric analysis. To perform stereotopometric analysis of the facial skeleton, we used the method developed by us to construct the base planes, which are centered at the reference point of the coordinate system. The latter is located between the trabecular and parachordal parts of the skull in the projection of the sagittal basal plane between the round hole. This point is recommended as a centering point in craniological studies for two reasons. The first confirms the results of observations of D.E. Lieberman, C.F. Ross, M.J. Ravosa (2000). Thus, all their

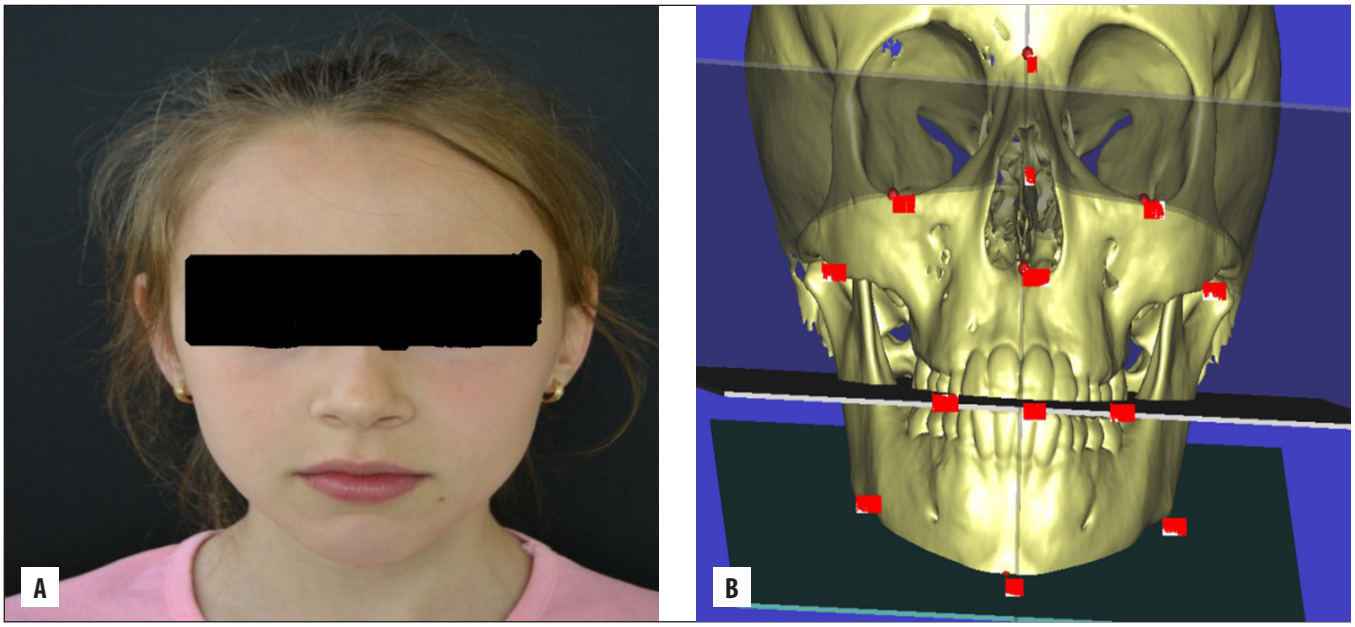

Fig. 1. A) Patient, 9 years old, oral habit leaning on her left arm; B) Computer reconstruction of the skull. Patient, 9 years old, oral habit leaning on her left arm

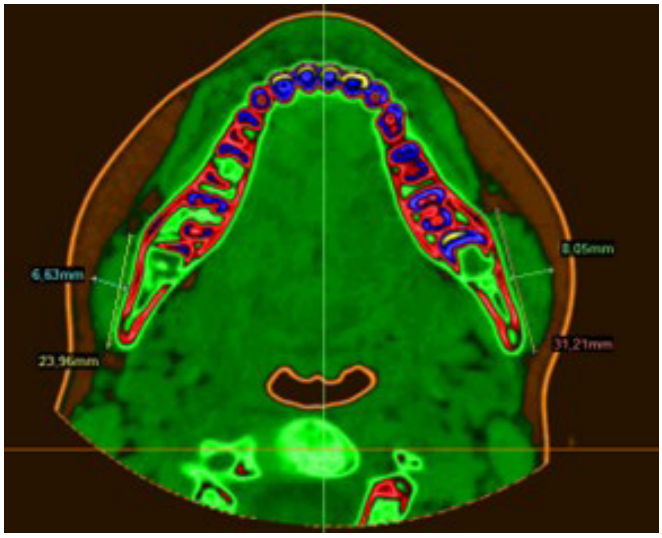

Fig. 2. SSD computer reconstruction of the skull, masticatory muscles disproportion. Patient, 9 years old, oral habit leaning on her left arm

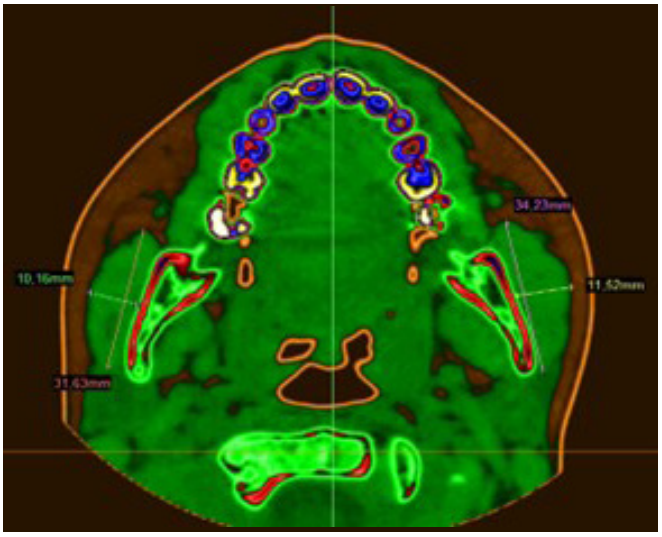

Fig. 3. SSD computer reconstruction of the skull, masticatory muscles disproportion. Patient, 9 years old, oral habit leaning on her left arm

studies of cranial growth processes point to the center of the basicranium (oval area near the body of a cuneiform bone), which reaches its final size and shape faster than other parts of the skull because all vital cranial nerves, vessels perforate the skull base in this area. The second reason is the transformation of the bony platform of the cuneiform bone under the influence of the growth of brain structures in contrast to the center relative to which the transformation occurs. To identify anthropometric points, we use standard anatomical zones defined in craniology[14, 13].

In order to determine the position of the upper jaw in the skull, we introduced the point of least variability of the upper jaw. The above navigation index was recorded by us in the center of the perpendicular dropped from the point of nasion to the middle of the base of the upper jaw. That is, this is the point of the center of the triangle of the upper jaw formed by the points N, PNS, ns. Measurement of angular and linear parameters is carried out automatically after marking the above anthropometric points. E. Martin's method was taken as a basis. Statistical processing of the results was performed using a personal computer using the software package Statistica 8.0. Data distribution was assessed using the Kolmogorov-Smirnov test of normality. Mean values and standard errors were calculated for continuous variables. Correlation between parameters was analyzed using Spearman's correlation

Table I. Parameters of linear cephalometric parameters in the control group and patients aged 9 to 12 years with acquired lower and upper micrognathia

№	Measured Indicator	Pathology under study		
		Upper micrognathia	Lower micrognathia	Norm
1.	N – Se	55.76±1.213 p<0.05	58.36±0.905 p>0.05	58.73±0.828
2.	Mart.63 Biom G2	22.58±1.253 p<0.02	25.78±0.682 p>0.05	25.91±0.586
3.	The distance between greater palatine foramen	22.46±0.989 p<0.007	25.47±0.765 p>0.05	25.91±0.592
4.	(ns) or VPOK – (pns)	34.69±0.77 p<0.05	37.05±0.924 p>0.05	37.33±0.915
5.	The position of the upper jaw in the skull from the point «0».	Y=34.85±1.185 p<0.02	Y=38.15±0.485 p>0.05	Y=38.11±0.625
6.	The position of the upper jaw in the skull from the point «S»	Y=41,75±1,295 p<0,001	Y=47,18±0,525 p>0.05	Y=47,02±0,551
7.	The position of the point «0»	Y=7.1±0.632 p<0.01	Y=9.01±0.465 p>0.05	Y=9.23±0.464
8.	PNS – ppw	6.34±0.581 p<0.0000006	11.68±0.684 p>0.05	12.36±0.624
9.	Ba – PNS	27.89±1.043 p<0.0000009	36.90±0.899 p>0.05	37.28±0.851
10.	T1	13.62±0.600 p>0.05	14.32±0.40 p>0.05	15.11±0.544
11.	P2	5,35±0,504 p<0.00002	8,48±0,478 p>0.05	9,40±0,663
12.	P3	6.21±0.481 p<0.0000003	12.34±0.80 p>0.05	13.76±0.815
13.	T2	22.53±0.548 p<0,05	24.59±1.09 p>0.05	25.23±0.876
14.	V	23.20±1.209 p<0.003	29.02±1.112 p>0.05	28.51±1.115
15.	N – SpP (Mx – Pl)	33.66±0.849 p<0.001	39.18±1.153 p>0.05	38.73±1.155
16.	Mart.60	34.62±1.174 p<0.05	39.03±0.904 p>0.05	38.87±1.197
17.	Mart.61	49.84±1.603 p>0,05	52.1±0.974 p>0,05	51.66±0.945
18.	Mart.55.Biom NH'.	32.33±0.794 p<0.05	36.5±1.216 p>0.05	36.45±1.447
19.	Mart.54.Biom NB.	18.06±0.797 p<0,04	20.06±0.434 p>0,05	20.26±0.591
20.	Mart.43(1) Biom IOW.	86.97±3.13 p>0.05	81.87±1.242 p>0.05	80.32±1.409
21.	Height of Nasion (N) above the line connecting the points fmol and fmor	13.64±0.881 p>0.05	15.68±0.653 p>0.05	14.54±0.985
22.	Mart.51a Biom O1'L.	33.97±0.844 p>0.05	33.32±0.477 p>0.05	32.98±0.528
23.	Mart.49a Biom DC.	20.46±1.132 p>0,05	18.06±0.569 p>0,05	18.43±0.814
24.	The depth of the orbita	34.82±1.262 p>0,05	36.12±0.53 p>0,05	35.49±0.694
25.	Determination of the symmetry of the medial edge of the orbita	8.33±0.637 p>0,05	8.66±0.307 p>0.05	8.36±0.419
26.	Mart 52.Biom.O2L.	31.26±0.908 p>0,05	31.54±0.596 p>0.05	30.24±0.751
27.	Mart.46.Biom GB.	66.65±1.782 p>0,05	67.82±1.343 p>0.05	68.03±1.676
28.	Mart.40	67.85±1.109 p<0.01	73.97±1.556 p>0.05	74.21±1.603
29.	Mart.48. Biom.G'H.	51.76±1.512 p>0.05	54.41±1.48 p>0,05	54.37±1.597
30.	Mart.5	85.14±1.143 p>0.05	86.71±1.553 p>0.05	87.19±1.632
31.	Mart.68.Biom Cp1.	48.51±1.997 p>0.05	42.64±1.491 p<0.003	49.41±1.541
32.	Biom. pg go straight length from angles	58.49±2.245 p>0.05	52.22±2.884 p<0.04	59.46±1.913
33.	The length of the body of the lower jaw (teleradiology graphic)	45.49±1.903 p>0.05	41.27±1.365 p<0.01	46.82±1.382

Notes: p – significance in compared groups during follow-up period

Table II. Parameters of linear cephalometric parameters in the control group and patients aged 9 to 12 years with acquired lower and upper micrognathia

№	Measured Indicator	Pathology under study		
		Upper micrognathia	Lower micrognathia	Norm
1.	Mart.70.Biom. Rl.	33.93±2.045	28.94±1.641	35.02±2.074
		p>0.05	p<0.04	
2.	Distance fromdistal point fragment to projection of the articular fossa	Jaw branch available	Jaw branch present	Jaw branch present
3.	The height of the branches of lower jaw MT2 (teleradiography)	33.68±2.068	34.86±1.591	33.46±2.143
		p>0.05	p>0.05	
4.	Total mandibular length	80.21±3.036	72.41±3.564	81.44±2.676
		p>0.05	p<0.04	
5.	Distance from Pg to the projection of the articular fossa	Jaw branch available	Jaw branch present	Jaw branch present
6.	PNS – ppw (teleradiography)	5.84±0.482	12.54±1.334	12.49±1.271
		p<0.00001	p>0.05	
7.	Ba – PNS (teleradiography)	27.51±1.108	34.84±0.895	34.86±0.782
		p<0.00001	p>0.05	
8.	T1 (teleradiography)	12.68±0.675	12.81±0.531	12.55±0.505
		p>0.05	p>0.05	
9.	P2 (teleradiography)	5.83±0.413	9.05±0.674	9.34±0.666
		p<0.001	p>0.05	
10.	P3 (teleradiography)	6.35±0.239	10.85±1.273	10.78±1.163
		p<0,0002	p>0,05	
11.	T2 (teleradiography)	20.76±0.829	23.99±0.988	23.51±0.59
		p<0.01	p>0.05	
12.	V (teleradiography)	21.14±0.921	28.38±0.905	27.10±0.898
		p<0,00009	p>0,05	
13.	N – SpP (Mx – Pl) (teleradiography)	33.57±2.238	38.86±1.078	38.53±1.231
		p>0.05	p>0.05	

Notes: p – significance in compared groups during follow-up period

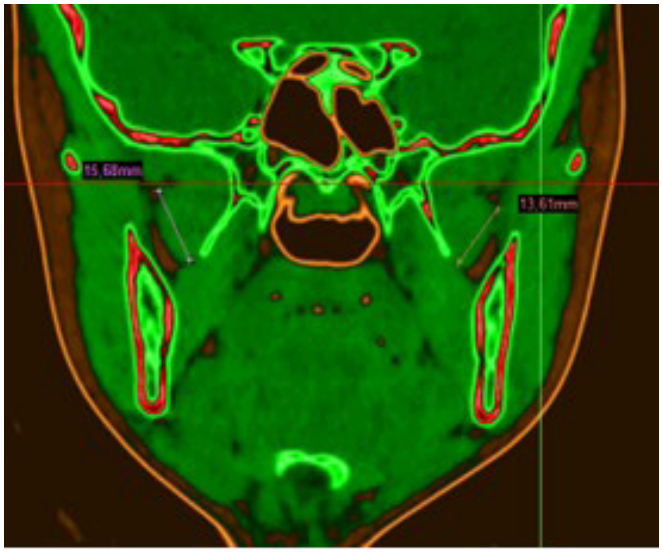

Fig. 4. SSD computer reconstruction of the skull, masticatory muscles disproportion. Patient, 9 years old, oral habit leaning on her left arm

coefficient and tested for significance. Significance was set at $p < 0.05$[15].

RESULTS

The results of a secret survey showed that 98.3% of the surveyed patients (59 people) have bad oral habits (supporting the head with hands – 53 people, sitting in front of a monitor with open mouth – 4 patients, keeping fingers in the mouth, pencils – 2 people).

In the comparison group, the results of the photo-protocol corresponded to the picture that accompanied the main diagnosis. 41.7% (25/60) of patients in the main group and 6 people in the comparison group were diagnosed with muscular hypertonia musculus oblicularis oris, while in the norm group there were no person this such pathological condition (p<0.01). In the presence of hypertension in patients, the dentition

Table III. Angular cephalometric parameters in the control group and patients aged 9 to 12 years with acquired lower and upper micrognathia

№	Measured Indicator	Pathology under study		
		Acquired upper micrognathia	Acquired lower micrognathia	Norm
1.	Angle F or front angle	71.59±1.322 p<0.01	78.05±1.257 p>0.05	77.81±1.372
2.	The position of the plane of the Frankfurt horizontal	Within the axial base plane	2,98±0,238	Within the axial base plane
3.	Position of the plane of the base of the upper jaw	Within the axial base plane	5,44±0,611	Within the axial base plane
4.	Position of the mandibular plane in the transverse plane	Within the axial base plane	5.62±0.79	Within the axial base plane
5.	The position of the sagittal plane to point A	Within the sagittal base plane	3.25±0.561	Within the sagittal base plane
6.	The position of the sagittal plane to the point Me	Within the sagittal base plane	8.05±1.161	Within the sagittal base plane
7.	Position of the plane Zml, Zmr, ANS	Within the axial base plane	4.38±0.49	Within the axial base plane
8.	The degree of inclination of the base of the upper jaw (Mx-Pl) in the sagittal plane	3.97±1.224 p>0.05	3.64±1.012 p>0.05	4.22±0.942

Notes: p – significance in compared groups during follow-up period

Table IV. Angular cephalometric parameters in the control group and patients aged 9 to 12 years with acquired lower and upper micrognathia

№	Measured Indicator	Pathology under study		
		Acquired upper micrognathia	Acquired lower micrognathia	Norm
1	Mart.77	135.6±1.263 p>0.05	137.1±1.489 p>0.05	137.94±1.469
2.	Position of the plane of the entrance to the orbita	75.14±1.277 p>0.05	77.36±1.244 p>0.05	76.93±1.177
3.	Position of the lateral wall of the orbita	36.41±1.822 p>0.05	37.56±0.892 p>0.05	37.42±0.893
4.	Position of the medial wall of the orbita	8.01±0.542 p<0.01	10.95±0.702 p>0.05	11.13±0.725
5.	Zigo-maxillary angle	110.36±1.575 p>0.05 p>0.05	109.58±1.378 p>0.05 p>0.05	108.74±1.326
6.	Angle N	53.52±1.271 p<0.01	57.93±1.241 p>0.05	58.34±1.231
7.	Angle A	84.62±1.545 p>0.05	83.64±1.298 p>0.05	83.84±1.411
8.	Angle B	37.31±0.594 p>0.05	38.41±0.776 p>0.05	38,13±0.904

Notes: p – significance in compared groups during follow-up period

narrows and shortens. This pathological condition is one of the etiological factors in the development of dental anomalies, in particular, the accumulation of teeth in the frontal jaw. Hypotonia of the circular muscles of the mouth was diagnosed in 15.0% (9/60) of patients in the main group and in 2 people in the norm group. Hypotonia is one of the etiological factors in the development of medial occlusion. At the time of the breath test, only 65.0% (39/60) had a positive test bilateral, indicating nasal breathing and proper

sinus formation. Other patients had nasal breathing disorders and were referred to an otorhinolaryngologist. The results of the clinical study were confirmed by cephalometric analysis, which indicated impaired formation of the maxillary sinuses (unilateral or / and bilateral) in those patients who do not have nasal breathing due to the anatomical structure of the nasal passages or inflammation in the sinus. Therefore, we recommend that you include these indicators as mandatory – in the diagnosis of maxillomandibular pathology.

According to the data of the 3D cephalometric examination presented in tables I, II in patients aged 9-12 with acquired anomalies of jaw development revealed disproportions distinctive of the gnathic part of the facial skeleton. Comparative analysis of maxillofacial parameters presented in patients with acquired upper micrognathia showed the presence of shortening to 34.69±0.77 mm ($p<0.05$) of the length of the base of the upper jaw (ns) or VPOK – (pns), which was reflected in the presence of mesial occlusion and typical of this type violation of the profile of the face, namely the depression of the upper lip and its base. The above changes were also confirmed by reducing the facial angle F to 71.59±1.322 ($p<0.01$). Shortening of the base of the upper jaw and reduction of the facial angle was combined with a change in the ratio of the chin bones and alveolar process of the upper jaw, which was reflected in the increase to 110.36±1.575 ($p>0.05$) zygo-maxillary angle. This type of disproportion in patients with acquired upper micrognathia was reflected in the change of facial profile: smoothness and flattening of the relief of the chin bones and occipital areas. Clinical examinations were confirmed by the results of cephalometric analysis. These patients have oral habits (sucking the tongue and / or fingers, sleeping with the mouth open), hypotonia of the circular muscles of the mouth, lack of new breathing. Patients with genetic factors in the development of medial occlusion were not included in the study.

Comparative analysis of the parameters of the facial skeleton, presented in tables I-IV in patients with acquired lower micrognathia showed the presence of malformations of its lower third. The expressed disproportions, as a rule, were noted in disturbance of development of both one, and symmetrically of two parties of a lower jaw. In the first case, a significant underdevelopment of the mandibular branch was combined with the existing bone ankylosis of the temporomandibular joint. In the second case, a significant symmetrical shortening of the mandibular branches was usually combined with intact temporomandibular joints. In both nosological units there was a shortening to 34.86±1.591 mm ($p>0.05$) in the height of the mandibular branch. The latter type of pathology was usually combined with a reduction of the projection length parameter from the corners, shortening to 41.27±1.365 mm ($p<0.01$) of direct length from the corners and reducing to 72.41±3.564 mm ($p<0.04$) of the total mandibular length. The above parameters were confirmed by distal occlusion and their characteristic facial profile, namely the beveled type of facial configuration silt in which the lower third of the face is shortened, the chin is shifted to the buttocks – "bird's face type", the lower lip is turned out, on which in most cases the upper incisors are located, the labial fossa is extremely well expressed, the lips do not close. The results of cephalometric analysis were confirmed by a photoprotocol, which on all indicators traced the shortening of the branches of the mandible. Such patients reported having an oral habit of leaning on their chin with their hands or sucking / biting their lower lip. One-sided disproportions are characterized by a violation of symmetry, which was confirmed by a shift of the sagittal plane to 3.24±0.557 compared with the norm. With such anomalies it is indeed important to assess the masticatory muscles and symmetrical areas of the face. There is a decrease in the thickness of the masticatory muscle, lateral and medial pterygoid muscles on the side where the patient has a habit of supporting the head. A significant positive correlation ($r_{x,y}$ = 0,85±0,14) was found between the presence of oral habit and acquired maxillomandibular anomalies ($p<0.05$).

We offer the results of a study of patient K., 9 years old with existing acquired deformities in the maxillofacial area. Diagnosis: acquired deformity of the lower jaw on the left side. According to computed tomography, there is a violation of the symmetry of the facial skeleton due to the displacement of the lower jaw to the right. The articular head of the left temporomandibular joint is displaced to the front (Fig. 1). No changes in the base of the skull were detected.

Analysis of the thickness of the masticatory muscles showed a difference between the left and right sides. The difference was also observed in the lateral pterygoid muscles (Figs. 2, 3, 4).

DISCUSSION

3D cephalometric analysis helped us identify the relationship between the presence of oral habit and acquired maxillomandibular anomalies. Our resurch has proven that 3D cephalometric analysis helps the orthodontist to properly examine the patient to make a correct plan of complex treatment and has a great ad-

vantage over all 2D diagnostic methods. Scientists also prefare 3D cephalometric analysis over 2D diagnostic methods [14, 2]. However some of them still use 2D diagnostic methods [1]. The results of our study showed the importance of such analysis, because thanks to it we can study the condition of the bones of the facial skull, temporomandibular joints, muscular system. In the process of studying the bones of the skull base, the doctor can determine whether the maxillomandibular anomaly and deformity is congenital or acquired and prove the relationship between the bad oral habit and the existing acquired deformity of the maxillofacial area.

Systematic use of oral habits causes constant traumatic effects on the bone structure and muscular system, and as a result deforms the affected area. We have found significant positive correlation ($r_{x,y}$= 0,85±0,14) between the presence of oral habit and acquired maxillomandibular anomalies ($p<0.05$). The results of clinical research and cephalometric analysis obtained by us allow us to do not reject the hypothesis of the theory of the functional matrix. The genetics of the organism play a major role in the formation of this matrix, but the long-term influence of physical forces plays a prominent role in the phenotype of bone structure. In the main group of patients, such a mechanical factor was the action of an oral habit that affected the phenotype, namely the maxillofacial area with the subsequent development of acquired deformities. The results of 3D cephalometry confirm this theory by the presence of bone deformity and thinning of the muscles from the side of the traumatic factor and causes compensatory muscle hypertrophy on the opposite side. The results of our study are confirmed by the results of other authors, who emphasize that physical forces play an important role in modulating cell function and shaping tissue structure and, as a result, start the process of mechanotransduction. Cells convert physically induced signals into biochemical reactions, are critical for mediating adaptation to mechanical stress in connective tissues [12, 6, 10, 11]. In this way, body tissues adapt to the effects of physical factors, triggering all the processes of adaptation of bone to the traumatic factor, namely the mechanisms of bone transduction. The bone is «tuned» to the exact frequency of skeletal muscle activity on the part of the factor. If the muscle develops, then the bone «does not see» the need. Everything in the human body is interconnected, but it is not always possible to convey this information to the patient.

CONCLUSIONS

1. Our resurch has showerd the relationship between the presence of oral habit and acquired maxillomandibular anomalies: 98.3% of patients with maxillomandibular anomalies and acquired deformities have oral habits. There is significant positive correlation ($r_{x,y}$= 0,85±0,14) between the presence of oral habit and acquired maxillomandibular anomalies ($p<0.05$). Results of radiological research and 3D cephalometric analysis has shown that the patients have disturbances in the bone apparatus and muscle structure on the side of the deformation (muscle thinning) and compensatory muscle hypertrophy on the opposite side. In the patients no changes were detected from the base of the skull, which indicates that the cause of the deformation is external factors, and not genetic syndromes like in congenital deformities. Radiological research methods help the orthodontist to identify a range of interrelated etiological factors in the development of acquired anomalies of the maxillofacial area, simplify a number of diagnostic manipulations and make the right plan for complex treatment.
2. 3D cephalometric analysis should be included in the main methods of differential diagnosis between acquired and congenital deformities of the maxillofacial area. It helps doctor to find etiological reason the maxillomandibular anomaly (oral habit or genetic defects of development).

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ORIGINAL ARTICLE

VENOUS THROMBOEMBOLISM – PECULIARITIES OF COURSE IN EMERGENCY SURGERY DURING COVID-19 PANDEMIC

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ABSTRACT

The aim: To perform a comparative analysis of VTE in patients with acute surgical abdominal pathology before and during the COVID-19 pandemic.

Materials and methods: Retrospective study covered 53062 patients operated in the surgical clinic (Lviv city emergency hospital) in 2000-2019. Prospective analysis was based on the results of treatment of 546 patients operated at the same surgical clinic from April 2020 (1st surgical patient with COVID-19) till December 2021. The study analyzed 48 (8.8%) patients operated for acute abdominal pathology and confirmed diagnosis of COVID-19.

Results: In the 1st group, heparin prophylaxis was used in 42.3% of patients, of which non-fractionated heparin were used in 58.6% and low molecular weight heparin – in the remaining patients. From 2020 to 2021, pharmacoprophylaxis was used in 84.5% of cases, of which 67.2% – low molecular weight heparins, 20.1% – non-fractionated heparins and 12.7% – modern oral anticoagulants. The results were unexpected: with a significant increase in the venous thromboembolism prevention in the 2nd group, a decrease in the number of episodes of thromboembolic complications was not observed. In contrast, pulmonary artery embolism was recorded in 10.6% of patients in the 1st group and 23.5% – in 2nd group, which is a 2.2-fold increase in fatal cases of venous thrombosis ($p<0.05$) in patients with COVID-19.

Conclusions: Increase of mortality due to pulmonary artery embolism more than twicefold in patients with COVID-19 operated for acute surgical abdominal pathology is an objective evidence of a potentiated, uncontrolled risk of venous thromboembolism and requires further in-depth study.

KEY WORDS: venous thrombosis and embolism, COVID-19, thromboembolic complications, acute surgical abdominal pathology

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INTRODUCTION

Prediction and prevention of post-operative complications remains one of the most pressing problems of clinical surgery. Venous thrombosis and embolism (VTE) is a complication, which differs from others by the particular severity of the course, complexity of diagnosis and treatment and high mortality. The sharp rise in venous thromboembolism (VT) in the context of the 2019 COVID-19 pandemic attracts increasing attention of experts around the world [1-4]. According to current data, about a third of patients' deaths in the postoperative period are due to VTE [1, 5, 6]. Clinicians [1, 2, 7] report that 71.4% of patients who died of COVID-19 were diagnosed with multifocal thrombosis as a manifestation of uncontrolled hypercoagulation. Although thromboembolic complications (TEC) are still considered to be the most favorable in the context of prevention, studies of last 2 years have raised the question of assessment of the real risk of VTE in surgical patients with severe respiratory syndrome caused by SARS-CoV.

THE AIM

To perform a comparative analysis of VTE in patients with acute surgical abdominal pathology before and during the COVID-19 pandemic.

MATERIALS AND METHODS

Retrospective analysis of the results of treatment of patients, who underwent surgery for acute surgical abdominal pathology before the COVID-19 pandemic (1st group) and prospective analysis – for the period from April 2020 to December 2021 (2nd group) were done.

The necessity of research was due to following: 1) from April 2020, hospitals began to admit surgical patients with concomitant COVID-19; 2) during the 2nd and 3rd quarters of 2020, there was an increase of VTE in patients with acute surgical abdominal pathology; 3) in early 2021, an urgent need for detailed study of TEC in the clinic and the development of differentiated approaches to specific prevention of VT; 4) rising number of reports about atypical localization of thrombosis of

veins (portal, mesenteric, splenic, renal etc.) in surgical patients with severe COVID-19-induced respiratory syndrome.

Retrospective study covered 53062 patients operated in the surgical clinic (Lviv city emergency hospital) in 2000-2019. Women slightly predominated (53.4%). The mean age of patients was 56.2±11.5. In emergency mode, 53547 interventions were performed for acute diseases of the abdominal organs (appendicitis – 20.1%, cholecystitis – 18.2%, bowel obstruction – 19.5%, incarcerated hernia – 10.8%, perforated gastric or duodenal ulcer – 12.3%, destructive pancreatitis – 3.4%, other acute surgical pathology – 5.5%) and abdominal trauma (10.2%).

After surgery, 1673 patients (3.2%) died, mainly from purulent-septic complications or multiple organ failure. Pulmonary artery embolism (PAE) occurred in 178 patients (10.6%) on 7.4±3.2 days after emergency surgery. In 12 patients (6.7%), PAE was clinically subacute and its intensive therapy proved to be effective. Clinical and radiological signs of pulmonary infarction appeared in 8 (4.5%) patients 3-4 days after PAE. Acute sudden heart and lung failure caused death of 101 (56.8%) patient despite resuscitation. In 65 (36.5%) patients with PAE, cause of death was revealed only at autopsy.

Prospective analysis was based on the results of treatment of 546 patients operated at the same surgical clinic from April 2020 (when 1st surgical patient was diagnosed with COVID-19) till December 2021. Men slightly prevailed – 52.7%. Age of patients ranged from 21 to 85 (mean – 62.1±21.2). Acute intestinal obstruction was the indication for surgery in 140 (25.6%) patients, 86 of them (15.8%) had obstructive colon cancer, acute appendicitis – 120 (22.0%), incarcerated hernia – 105 (19.2%) (of which intestinal resection was done in 4.7%), acute cholecystitis – 89 (16.3%), perforated ulcer – 68 (12.5%), perforated diverticulitis of the descending and sigmoid colon – 14 (2.6%) and segmental small bowel necrosis – 10 (1.8%).

The study analyzed 48 (8.8%) patients operated for acute abdominal pathology and confirmed the diagnosis of COVID-19. Positive polymerase chain reaction (PCR) for identification of SARS-CoV-2 was confirmed in 83.3% of patients, express test for antigen was positive only in 17.7% of cases. There were 37 (77.1%) patients in the specialized Intensive care unit (ICU) for patients with COVID-19, of whom 17 (45.9%) required invasive breathing support, average bed stay was 7.6±3.2 days. On days 3.8±1.9, 17 (35.4%) post-surgical patients died, autopsy was performed in 15 (88.2%). Causes of death were: multiple organ failure (35.3%), heart and respiratory failure (29.4%), PAE (23.5%) and purulent-septic complications (11.8%).

In 1st group, 37.1% of patients were diagnosed with peritonitis of various spread: local delimited in 31.2%, local non-delimited – 25.7%, diffuse in – 43.1%. Mannheim peritonitis index (MPI) value was: I – 27.4%, II – 52.3% and III – 20.3%. Despite the fact that in the 2nd group there were more patients with diffuse peritonitis (49.1%) and cases with MPI value III were slightly predominant, no significant difference ($p>0.05$) was noticed between the indicators.

However, assessing the preoperative condition of patients according to the classification of American Society of Anesthesiologists, it was found that in 1st group class I was stated in 13.3% of patients, in 2nd – 9.8%; class II – in 26.2% and in 21.3% respectively; class III – in 28.4% and in 32.4% respectively; class IV – in 21.0% and in 25.2% respectively; class V – in 11.1% and in 11.3% respectively. The increase in the number of patients with class III and IV in the 2nd group was due to an increase in the incidence of disseminated intravascular coagulation and respiratory failure due to viral pneumonia and decreased cardiac ejection fraction due to overload of right part of heart caused by VTE.

Patients of both groups were individually stratified according to VTE risk levels according to the J. Caprini risk assessment scale [5, 8]. Low (1-2 points), moderate (3-4 points) and high (5 or more points) degrees of risk in the 1st group were found in 3.5%, 72.2% and 24.3% of patients, in 2nd – respectively, in 2.8%, 62.2% and 35.0% cases ($p<0.05$). All patients with moderate and high risk were given VTE drug prophylaxis according to the current recommendations and taking into account the peculiarities of COVID-19.

Pre-operative preparation both study groups consisted of correction of water-electrolyte balance, functions of major organs and systems, antibiotic prophylaxis or antibiotic therapy. All surgical interventions were performed within 24 h using general anesthesia in 87.5% of patients (endotracheal – 74.2%), regional– in 12.5%. The average duration of the operation was 98±51.8 min.

RESULTS AND DISCUSSION

Venous thrombosis and embolism is a complication in surgery being exceptional for the number of existing guidelines, consensuses, clinical protocols and standards of prevention [6, 9]. Nevertheless, with the threating pace of SARS-CoV-2 spread, global medicine had faced new, unknown challenges associated with VT, which are much more aggressive, dramatically worsening treatment outcomes. Assessing the danger, it was necessary to modernize clinical guidelines, mainly changing the concept of VTE risk assessment in patients

with COVID-19 and developing new diagnostic and preventive methods, adapted to current reality.

For comparative analysis, we stratified both study groups by degree of VTE risk in accordance with the 9th ACCP guideline. A distinct increase in the number of high-risk patients with TEC was seen in the 1st group from 24.3% to 35% ($p<0.05$), while in the 2nd this was due to an increase in the number of operations for complicated surgical pathology, significant increase in hospital stay and, consequently, prolongation of bed rest for more than 72 hours and the number of central vein catheterizations.

Analysis of the nature and effectiveness of specific VTE prevention was performed in both study groups. In the 1st group, heparin prophylaxis was used in 42.3% of patients, of which non-fractionated heparins (NFH) were used in 58.6% and low molecular weight heparins (LMWH) – in the remaining patients. From 2020 to 2021, pharmacoprophylaxis was used in 84.5% of cases, of which 67.2% – LMWH, 20.1% – NFH and 12.7% – modern oral anticoagulants. The results were unexpected: with a significant increase in the VTE prevention in the 2nd group, a decrease in the number of episodes of TEC was not observed. In contrast, PAE was recorded in 10.6% of patients in the 1st group and 23.5% – in 2nd group, which is a 2.2-fold increase in fatal cases of VT ($p<0.05$) in patients with COVID-19. The reasons for such a substantial increase in the incidence of VT are confirmed by preliminary data on the potentiated risk of VTE in patients with acute surgical abdominal pathology on the background of severe respiratory syndrome caused by SARS-CoV-2.

As well, this comparative analysis of groups' stratifications revealed a slight increase in the number of patients with a high risk of VTE in the 2nd (COVID-19) group. Widely known, in case of low degree risk it is advisable to limit the use of mechanical prophylaxis, while in moderate and high degrees of risk, the use of anticoagulants in appropriate doses is necessary [10]. Therefore, the application of the 8th edition of the ACCP guideline and local clinical protocols, especially developed for emergency surgery, will elevate the importance of pharmacological prevention of VTE. Giving priority to specific prophylaxis will optimize the solution to the problem of VTE in Ukraine, where most mechanical methods (graded elastic compression, intermittent pneumocompression, electrical muscle stimulation etc.) are mostly not available and non-specific prevention is reduced only to early activation of the patient after surgery.

Indicators of the timing of death after surgery in study groups deserve special attention. Thus, in the 1st group it equaled 7.4±3.2 days, in 2nd – 3.8±1.9, which may point upon a more aggressive course of thanatogenesis in patients with COVID-19 and a theoretical increase in the number of TEC in patients due to the progression of respiratory failure, which do not "live up" until PAE occurs.

CONCLUSIONS

1. Venous thrombosis and embolism are one of the most serious postoperative complications in emergency surgery, especially on the background of the COVID-19 pandemic.
2. Modern features of the problem of VTE in surgical patients with COVID-19 are the decrease in the proportion of patients with low risk of VTE and an increase in the number of patients with high risk of VTE.
3. Optimizing the solution to the problem of VTE in emergency surgery is seen in assessment of the real scale of TEC in patients with COVID-19 and widening of the indications for pharmacological prevention of VT.
4. Increase of mortality due to PAE more than twicefold in patients with COVID-19 operated for acute surgical abdominal pathology is an objective evidence of a potentiated, uncontrolled risk of VTE and requires further in-depth study.
5. Actual TEC values in surgical patients with COVID-19 may be underestimated due to shortening of the hospitalization-death period.

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ORIGINAL ARTICLE

ACCUMULATION OF HEAVY METALS IN THE COLON AND BLOOD OF PATIENTS WITH COLORECTAL CANCER

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ABSTRACT

The aim: To determine the accumulation of heavy metals, namely Cr, Cd, Cu, Pb, Zn by a tumour in correlation with unaffected colon tissue and blood of patients with colorectal cancer.

Materials and methods: The study is based on the results of observation of 180 patients with CRC. The following samples were taken from: the tumor, the colon and blood unaffected by the tumor – during the operative treatment of patients in the surgical department of the communal non-profit enterprise "Precarpathian Clinical Oncology Center of the Ivano-Frankivsk regional council" for 2018-2020.

Results: According to the obtained results, the blood of patients with CRC contains much less heavy metals: Pb – 8.6 and 9.3 times less than in the tumour and unaffected by cancer colon, respectively; Cr – 9 and 14.8 times less; Cd – 3.75 and 5 times less; Cu – 1.48 and 1.18 times less than in the tumour and unaffected colon, respectively. Besides, the content of Zn in the blood is 1.04 times higher than in non-cancerous tissue of the colon.

Conclusions: It can be stated that the highest content of Pb, Cr and Cd was recorded in non-cancerous tissue of the colon of patients with CRC; the highest content of Zn and Cu was revealed tumour tissue.

KEY WORDS: colorectal cancer, heavy metals, colon

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INTRODUCTION

Carcinogenesis of heavy metals (HM) occurs in two main ways: direct impact on the genetic apparatus of the cell, and due to the impact on the enzymatic systems. The danger of HM poisoning is due to their ability to accumulate. In the human body, heavy metals are accumulated on the surface of chromosomes and lead to structural modifications of nucleic acids.

A number of clinical and experimental studies have shown that some carcinogenic HMs are characterized by inhibitory effects on the growth of malignant tumours at certain concentrations [1-3].

According to a number of scientific studies, environmental contamination by heavy metals (HM) is in second place in terms of environmental hazards after pesticides pollution. The problem of increasing number of HMs in the environment has led to the intensification of the study of their impact on carcinogenesis; the available literature contains many studies devoted to carcinogenic effects of environmental pollution [4].

The accumulation of HMs in the external environment is due to their characteristic features: resistance to environmental factors, solubility in water medium, sorption by soil and plants. [5-7].

HMs are accumulated in tumour tissue in breast cancer. Besides, a correlation between HMs content in the tumour and habitual area contaminated by VMs has been found.

According to recent studies, there is a correlation between the content of chromium and lead in the blood of patients with colorectal cancer (CRC) and the duration of recurrence-free survival [8].

CRC is characterized by high heterogeneity of course of the disease and response to therapy, even within clinically homogeneous patient groups. From the standpoint of the above, there is a need for new prognostic factors [9-11].

THE AIM

According to the obtained results, the content of such heavy metals as Cr, Cu, Cd in the blood of patients with CRC is statistically significantly higher than in healthy people, while the content of Zn and Pb in the blood is lower.

Table I. Pb content in patients with CRC

Test material	Blood	Primary tumour	Colon without malignant neoplasm
Average value µg/g	0.03	0.26	0.28
Standard error	0.001	0.043	0.016

Table II. Cr content in patients with CRC

Test material	Blood	Primary tumour	Colon without malignant neoplasm
Average value µg/g	0,06	0,54	0,89
Standard error	0,001	0,114	0,150

Table III. Cd content in patients with CRC

Test material	Blood	Primary tumour	Colon without malignant neoplasm
Average value µg/g	0,04	0,15	0,20
Standard error	0,001	0,017	0,025

Table IV. Cu content in patients with CRC

Test material	Blood	Primary tumour	Colon without malignant neoplasm
Average value µg/g	1,37	2,03	1,61
Standard error	0,04	0,27	0,41

Table V. Zn content in patients with CRC

Test material	Blood	Primary tumour	Colon without malignant neoplasm
Average value µg/g	4,35	7,74	4,19
Standard error	0,07	0,66	0,85

With an increase of chromium in the blood of patients with CRC (regression coefficient is -22.2782) and lead (regression coefficient is -8.8742), there is a statistically significant lower recurrence-free survival.

The aim – To determine the accumulation of heavy metals, namely Cr, Cd, Cu, Pb, Zn by a tumour in correlation with unaffected colon tissue and blood of patients with colorectal cancer.

MATERIALS AND METHODS

According to a previous study, it was determined that in patients with colorectal cancer, the content of chromium in the blood is 2.2, cadmium is 3.4, and copper is 1.4 higher than in healthy people, while the content of lead and zinc in the blood of patients with colorectal cancer is lower by 3 and 1.4 times, respectively ($p<0.001$ – for chromium, cadmium, zinc and lead; $p<0.05$ – for copper). The prognostic assessment of heavy metals on recurrence-free survival in colorectal cancer patients was carried out (using the construction of a regression model) – with the content of chromium (regression coefficient -22.3) and zinc (regression coefficient 0.5), $p<0.05$, i.e., with an increase in the chromium content in the blood of patients and a decrease in zinc, a lower recurrence-free survival rate was observed. We think it is rather important to understand the distribution of the studied metals in the body of patients with colorectal cancer (CRC).

The study is based on the results of observation of 180 patients with CRC. The following samples were taken from: the tumor, the colon and blood unaffected by the tumor – during the operative treatment of patients in the surgical department of the communal non-profit enterprise "Precarpathian Clinical Oncology Center of the Ivano-Frankivsk regional council" for 2018-2020.

In order to understand the peculiarities of the accumulation and distribution of heavy metals in the body of patients with CRC, the following groups were formed for comparison:

- 157 patients with metal content in the blood;
- 39 patients with metal content of in the primary tumor;

To study the content of heavy metals in the colon unaffected by the tumor of CRC patients and to study the accumulation of metals in the colon, samples were taken from 12 persons out of 39 studied patients.

The database was formed using Ms Excel tables. Statistical processing of qualitative (categorical) data was carried out using the methods of non-parametric statistics and by determining the frequency of various clinical manifestations per 100 examined persons in

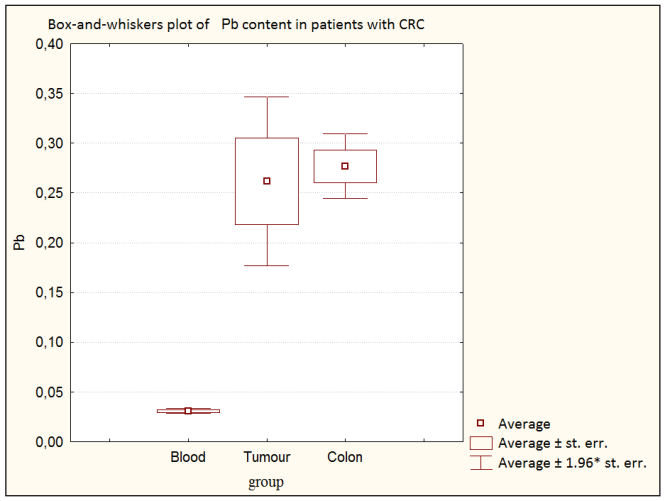

Fig. 1. Box-and-whiskers plot of Pb content in patients with CRC. According to calculations, p = 0.000000, which indicates the reliability of the data.

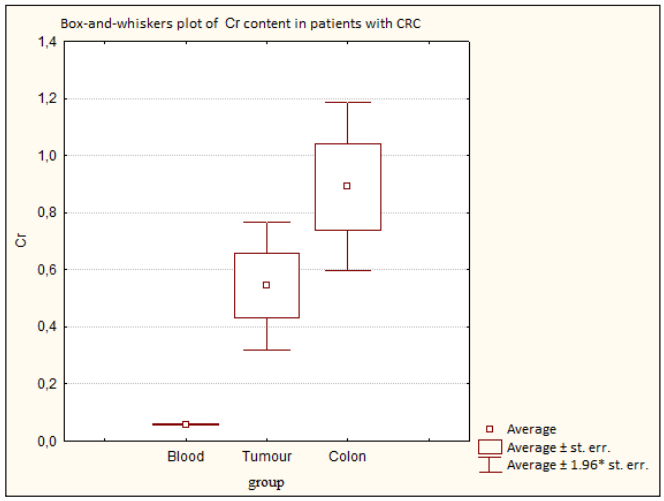

Fig. 2. Box-and-whiskers plot of Cr content in patients with CRC. According to calculations, p = 0.000000, which indicates the reliability of the data.

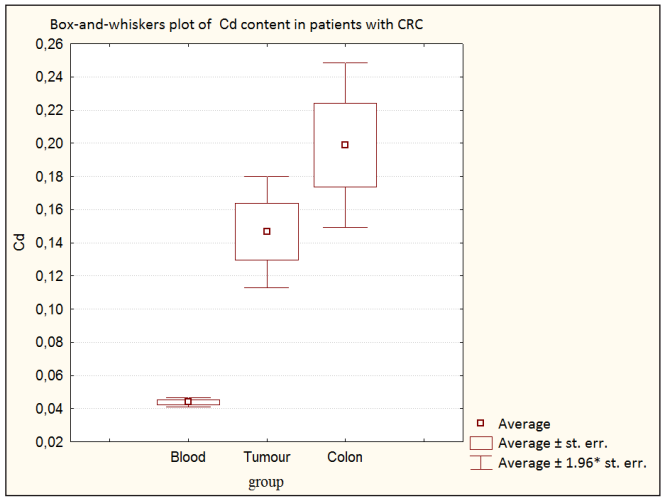

Fig. 3. Box-and-whiskers plot of Cd content in patients with CRC. According to calculations, p = 0.000000, which indicates the reliability of the data.

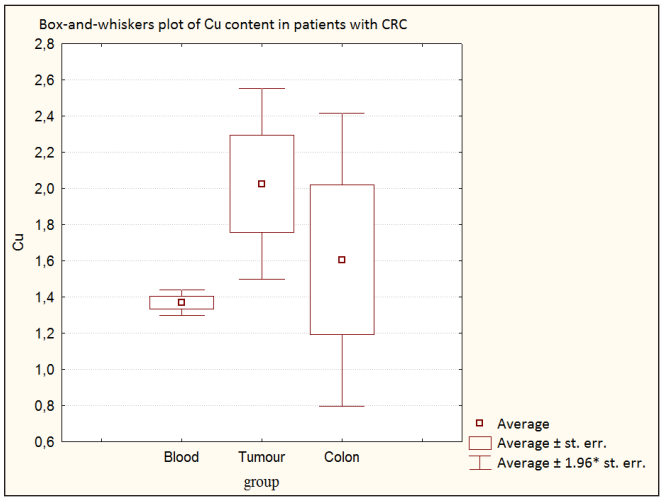

Fig. 4. Box-and-whiskers plot of copper content in patients with CRC. According to calculations, p = 0.000248, which indicates the reliability of the data.

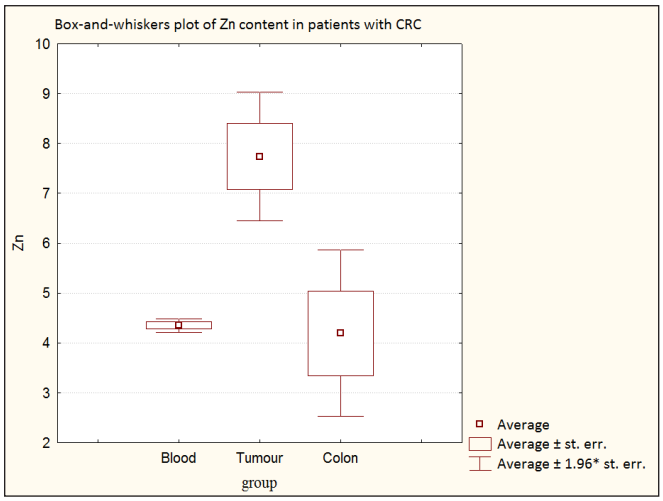

Fig. 5. Box-and-whiskers plot of zinc content in patients with CRC. According to calculations, p = 0.000000, which indicates the reliability of the data.

each group (in %).

The reliability (confidence limits) of the obtained indicators was confirmed by calculating the error (± m) for relative values.

Using the calculation of Pearson's chi-square ($\chi 2$) correspondence criterion, the reliability of the difference in frequency indicators in different observation groups was evaluated, as well as the null hypothesis was tested.

Variational and statistical analysis of the research to assess the degree of reliability of the results was carried out using a personal computer, Microsoft® Office Excel® 2007 and Statistica v.6 application programs (Statsoft Inc., USA).

Comparison of two fractions: two sets of objects that may have a random attribute A. Two samples of volumes nx and ny are formed from these sets. The number of objects with feature A in the first sample is a random

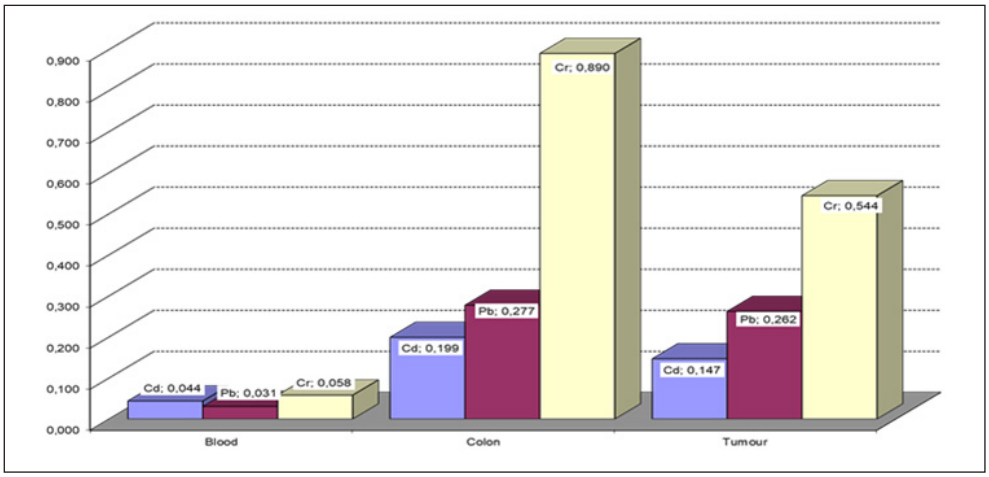

Fig. 6. Average values of Pb, Cr and Cd content in patients with CRC.

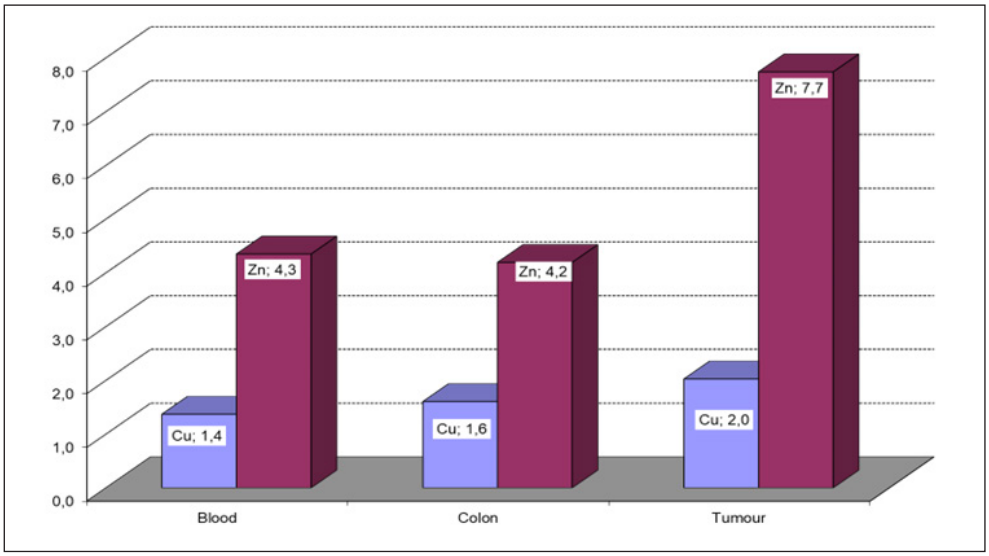

Fig. 7. Average values of Cu and Zn in patients with CRC.

variable X, and in the second – a random variable Y. It is believed that the random variables X and Y are independent and subject to the binomial distribution law with parameters (nx, px) and (nY, pY).

The indicators of the degree of correlation between the studied indicators, the relative risk (odds ratio) and the correlation coefficient were studied. One type of indicators shows a change in risk when moving from one discrete group to another; the other one indicates the regularity in the interaction of variables over the entire range of values. Mostly, relative risk and odds ratio are used to describe interaction between qualitative variables (measured on a nominal scale).

By determining the odds ratio, we can estimate the correlation between a certain result and a risk factor, as well as compare groups of subjects by the frequency of detection of a certain risk factor and determine its quantification.

The correlation coefficient is used to analyze interaction involving at least one quantitative or semiquantitative variable.

The influence of the studied metals in the patients' blood on the duration of the relapse-free period (in months) was determined based on the construction of the regression model: y=EXP(4.224-0.343*Cu+0.470*Zn-2.243*Cd-8.874*Pb-22.278*Cr).

RESULTS

According to a previous study, the content of heavy metals, namely: Cr, Cd, Cu, Pb, Zn in the colon of patients with CRC and healthy patients was studied. The following results were obtained: the content of heavy metals, namely Cr – 28.7 times higher ($p = 0.000044$), Pb – 2.77 times higher ($p = 0.000000$) and Cd – 3.2 times higher ($p = 0.000117$) in unaffected colon of patients with CRC than in the colon of healthy patients; whereas for Zn and Cu the obtained data are not statistically significant ($p = 0.303704$ and $p = 0.095517$, respectively).

COMPARISON OF LEAD ACCUMULATION BY TUMOUR, UNAFFECTED BY CANCER COLON AND BLOOD OF PATIENTS WITH CRC

In patients with CRC, unaffected by cancer tissue of the colon is 1.1 times more prone to Pb accumulation compared to the cancer tumour. Much lower metal

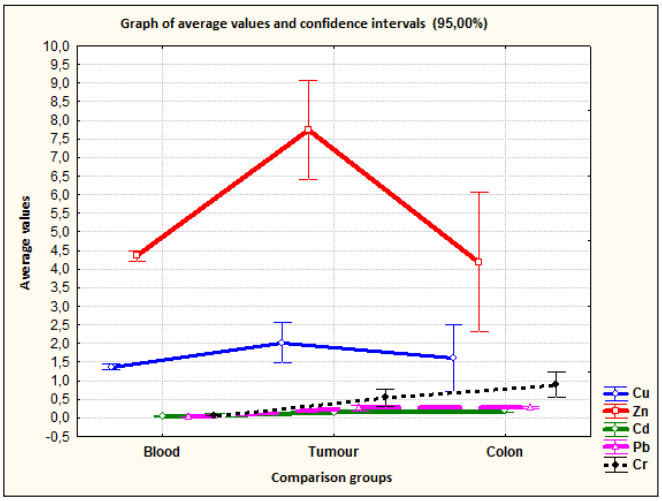

Fig. 8. Average values and confidence intervals of Cr, Cd, Cu, Pb, Zn content in patients with CRC.

levels are detected in the blood of patients: 8.6 and 9.3 times less than in the tumour and unaffected by cancer colon, respectively (Table I, Fig. 1).

COMPARISON OF CHROMIUM ACCUMULATION BY TUMOUR, UNAFFECTED BY CANCER COLON AND BLOOD OF PATIENTS WITH CRC

According to the obtained data, unaffected tissue of the colon is 1.65 times more prone to accumulation of Cr compared to the cancer tumour. Much lower metal levels are detected in the blood of patients: 9 and 14.8 times less than in the tumour and unaffected by cancer colon, respectively (Table II, Fig. 2).

COMPARISON OF CADMIUM ACCUMULATION BY TUMOUR, UNAFFECTED BY CANCER COLON AND BLOOD OF PATIENTS WITH CRC

In patients with CRC, unaffected by cancer tissue of the colon is 1.3 times more prone to Cd accumulation compared to the cancer tumour. Much lower metal levels are detected in patients' blood: 3.75 and 5 times less than in the tumour and unaffected by cancer colon, respectively. (Table III, Fig. 3).

COMPARISON OF COPPER ACCUMULATION BY TUMOUR, UNAFFECTED BY CANCER COLON AND BLOOD OF PATIENTS WITH CRC

In patients with CRC, the tumour is 1.26 times more prone to Cu accumulation compared to unaffected by cancer tissue of the colon. A lower level of metal is detected in the blood of patients: 1.48 and 1.18 times less than in the tumour and colon without neoplasm, respectively (Table IV, Fig. 4).

COMPARISON OF ZINC ACCUMULATION BY TUMOUR, UNAFFECTED BY CANCER COLON AND BLOOD OF PATIENTS WITH CRC

In patients with CRC, the tumour is 1.85 and 1.8 times more prone to Zn accumulation compared to unaffected by cancer tissue of the colon and blood, respectively (Table V, Fig. 5).

Having analyzed the obtained data, we can state that levels of lead, chromium and cadmium in unaffected by cancer tissue of the colon were higher compared with these levels in a tumour (1.1 times – for Pb, 1.65 times – for Cr and 1, 3 times – for Cd). In all cases, much lower content of heavy metals was revealed in the blood of patients with CRC (Fig. 6).

The results of study showed that the levels of zinc and copper were higher in tumor tissue compared to unaffected by cancer colon tissue of patients with CRC (1.26 times – for Cu and 1.85 times – for Zn). The content of these metals in the blood and healthy tissue of the colon is at a relatively equal level.

It should be noted that zinc was mostly accumulated in the blood of patients with CRC. Its content in blood was 1.04 times higher in comparison with unaffected tissue of the colon (Fig. 7).

To summarize the obtained results, we made a graph of average values and confidence intervals of Cr, Cd, Cu, Pb, Zn content in blood, healthy colon tissue and tumour of patients with CRC (Fig. 8).

DISCUSSION

Despite the latest diagnostic methods, immunohistochemical studies, microsatellite instability and modern treatments based on the molecular profile of the tumour, the results of survival of patients with colorectal cancer remain unsatisfactory, which encourages scientists to continue studying this pathology. Patients with stage II colorectal cancer (CRC) are of particular interest, as the choice of adjuvant treatment for these patients depends on negative prognostic factors.

According to the NCCN recommendations, 2020, negative prognostic factors in patients with colorectal cancer include: poorly differentiated and undifferentiated tumour, lymphatic and perineural invasion, less than 12 examined lymph nodes, intestinal obstruction, and localized perforation. If these prognostic factors are present, adjuvant chemotherapy is recommended. However, the adjuvant therapy is not suggested if there

are only one or two negative prognostic factors.

Besides, there is evidence in the literature that negative prognostic factors are the following: young and senile age of patients, mutations in KRAS and BRAF, positive TS marker, CEA level above 25.0 ng/ml, microinvasive foci, morphometric parameters of coagulation tumour necrosis, infiltration of the tumour stroma by immunocytes, lymphocytic infiltrates, and the ratio of neutrophils to NLR>4 lymphocytes.

Recently, a lot of information has appeared in the literature on the impact of environmental pollution on the occurrence of cancer. According to a number of scientific studies, environmental pollution by heavy metals is in second place in terms of environmental hazard, after pollution by pesticides.

There is no study on the effects of heavy metals on the prognosis and course of colorectal cancer in the available literature.

Thus, the study of new prognostic factors will help clinicians in case of a negative prognosis to prescribe adjuvant therapy in a timely manner and prevent recurrence.

CONCLUSIONS

Therefore, it can be stated that the levels of Pb, Cr and Cd, revealed in patients with CRC, in unaffected by cancer tissue of the colon were higher than in the tumour (1.1 times – for Pb, 1.65 times – for Cr and 1.3 times – for Cd). Much lower levels of heavy metals were revealed in the blood of patients with CRC: Pb – 8.6 and 9.3 times less than in the tumour and colon without cancer, respectively; Cr – 9 and 14.8 times less than in the tumour and colon without cancer, respectively; Cd – 3.75 and 5 times less than in the tumour and colon without cancer, respectively.

When studying the accumulation of Zn and Cu, the highest metal content was recorded in tumour tissue compared to unaffected by cancer colon tissue of patients with CRC (1.26 times higher – for Cu, and 1.85 times higher – for Zn).

The content of Cu in the blood of patients: 1.48 and 1.18 times less than in the tumour and colon without cancer, respectively.

It should be noted that the content of Zn in the blood was 1.04 times higher than in healthy tissue of the colon.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

REACTIVE OXYGEN SPECIES GENERATION BY BLOOD LEUCOCYTES OF RATS WITH POLYCYSTIC OVARY SYNDROME UNDER THE CONDITIONS OF INTERMITTENT COLD EXPOSURE

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ABSTRACT

The aim of this study was to determine the level of ROS production by blood leukocytes of rats with PCOS under the conditions of intermittent cold exposure.

Materials and methods: In the study, 40 immature female rats of the WAG population at the age of 27 days with a body weight of 80-90 g were used. Five groups were formed (8 animals in each group). Group 1 was represented by intact rats that were not subjected to any manipulations. Group 2 was represented by rats that were injected subcutaneously with 0.2 ml of purified and sterilized olive oil daily for 25 days. Group 3 was represented by rats that were exposed to intermittent cold for 25 days. Group 4 was represented by rats that were modeled with PCOS. Group 5 was represented by rats, which were simulated PCOS against the background of intermittent cold exposure. ROS production was estimated in leukocytes isolated from rats of all groups by flow cytometry using the fluorescent probe of 2,7-dichlorodihydrofluorescein diacetate (H2DCFDA).

Results: The experimental study revealed an intracellular excessive production of ROS by leukocytes in rats with polycystic ovary syndrome. The use of intermittent cold exposure normalized the production of reactive oxygen species by leukocytes in rats with polycystic ovary syndrome.

Conclusions: The effectiveness of intermittent cold exposure, proven by the authors, allows recommending its use as one of the methods of prevention and treatment of the polycystic ovary syndrome.

KEY WORDS: leukocytes, rats, reactive oxygen species, polycystic ovary syndrome, intermittent cold exposure, oxidative stress

Wiad Lek. 2023;76(7):1670-1676

INTRODUCTION

Polycystic ovary syndrome (PCOS), the major endocrinopathy among reproductive-aged women, is not yet perceived as an important health problem in the world. It affects 4-20% of women of reproductive age worldwide [1]. This pathology is associated by hyperandrogenism, hirsutism, menstrual disorders, ovulatory dysfunction, polycystic ovaries and metabolic disorders [2, 3]. In the development of PCOS, according to many scientists, one of the key roles is played by oxidative stress, in which there is an increase in the production of reactive oxygen species (ROS) [4, 5].

It is now known that free radical reactions involving free radicals (a part of a molecule or a molecule having an unpaired electron) can lead to the formation of ROS and reactive nitrogen species. Oxidative stress reflects a disturbance in the balance between the formation and elimination of ROS. ROS include both free radical and non-free radical molecules, in particular hydrogen peroxide, hydroxyl radical, singlet oxygen, etc. [4-6]. In a healthy body, there is a balance between oxidant and antioxidant systems (enzymatic and non-enzymatic) [4]. The study of pathophysiological mechanisms of the oxidative stress development in PCOS is necessary for the development of modern effective methods of preclinical correction, prevention and rehabilitation of reproductive pathology.

In patients with PCOS, a combination of low-grade chronic inflammation, vascular endothelium damage, excessive production of ROS and oxidative stress development is common [4-8]. PCOS patients demonstrated significantly higher concentrations of circulating inflammatory cells, such as lymphocytes, neutrophils, eosinophils, monocytes etc. [7]. It is extremely important to search the sources, i.e. cells that can produce an excessive amount of ROS causing the development of PCOS.

THE AIM

The aim of the study was to determine the level of ROS production by blood leukocytes of rats with PCOS under the conditions of intermittent cold exposure.

MATERIALS AND METHODS

In the study, 40 immature female rats of the WAG population at the age of 27 days with a body weight of 80-90 g were used. Five groups were formed (8 animals in each group).

Group 1 was represented by intact rats that were not subjected to any manipulations.

Group 2 was represented by rats that were injected subcutaneously with 0.2 ml of purified and sterilized olive oil daily for 25 days.

Group 3 was represented by rats that were exposed to intermittent cold for 25 days. Intermittent cold exposure was modeled by keeping animals daily for 4 hours in a chamber where the light regime and temperature + 4°C were maintained. The animals were kept under normal conditions for the last 20 hours of the day. The animals were exposed to intermittent cold for 25 days.

Group 4 was represented by rats that were modeled with PCOS. PCOS was modeled by daily subcutaneous administration for 25 days of dehydroepiandrosterone (DHEA) at a dose of 8 mg per 100 g of animal body weight, dissolved in 0.2 ml of purified and sterilized olive oil.

Group 5 was represented by rats, which were simulated PCOS against the background of intermittent cold exposure. In this group, the methods for modeling of intermittent cold exposure and PCOS were similar to those used in groups 3 and 4.

Rats of all groups receiving the same diet were removed from the experiment by cervical dislocation on the 26th day. After decapitation of animals of groups 1-5 blood samples were collected to vacutainer tubes with ethylenediaminetetraacetic acid dipotassium salt (EDTA) (Guangzhou, China).

Preparation of samples (blood) for research on a flow cytometer according to the protocol took place at the Institute of Experimental and Clinical Medicine of the Kharkiv National Medical University (Ukraine).

The method used in this study for determining the generation of ROS in leukocytes was described by us in a previously published article [9].

Suspension of leukocytes was prepared. Blood from each animal lysed and washed twice with Pharmalyse solution (BD, USA) and phosphate-buffered saline. Blood samples in the amount of 100 µl were placed in a 12×75 mm polystyrene tube (Falcon, Mexico), and 2 ml of 1x FACSLyse solution (BD FACS™ Lysing, San Jose, USA) was added. We mixed and incubated in the dark at 23°C for 15 minutes. Then, for 5 min, centrifugation was carried out at 500 g. Supernatant liquid was discarded and 2 ml of sodium phosphate buffer was added.

Leukocyte suspensions were used for further evaluation of ROS levels in leukocytes. The fluorescent probe of 2,7-dichlorodihydrofluorescein diacetate (H2DCFDA) was employed for detection the intracellular ROS concentration. It is cleaved by intracellular esterases to form 2,7-dichlorodihydrofluorescein, which is transformed by ROS into highly fluorescent 2,7- dichlorofluorescein (DCF).

The flow cytometer BD FACSCanto ™ II (BD Biosciences, USA) was used. The mean fluorescence intensity (MFI) of DCF was analyzed using BD FACSDiva™software (Becton Dickinson, USA) for quantitative assessment of ROS production by leukocytes.

Statistical processing of the obtained data was performed using the Graph Pad Prism 5 program (Graph Pad Software, USA). The indicators were compared using the non-parametric Mann-Whitney U test. The results in groups were presented in the form of median (Me) and interquartile ranges. The differences at $p<0.05$ were considered statistically significant.

RESULTS

In groups 1-5, flow cytometry allowed us to assess the generation of ROS by analyzing the fluorescence inten-

Table I. The analysis of the fluorescence intensity of DCF in leukocytes of peripheral blood of rats of groups 1-5.

Group number	MFI of DCF in leukocytes	
	Median	[25% percentile; 75% percentile]
Group 1	407.1	[355.8; 425.9]
Group 2	416.5	[374.8;456.0]
Group 3	361.6	[297.2;409.5]
Group 4	539.4 [1,2,3]	[494.5; 632.4]
Group 5	377.0 [4]	[347.5; 404.8]

Note: [1] – significant ($p<0.05$) difference compared to the indicator of group 1; [2] – significant ($p<0.05$) difference compared to the indicator of group 2; [3] – significant ($p<0.05$) difference compared to the indicator of group 3; [4] – significant ($p<0.05$) difference compared to the indicator of group 4.

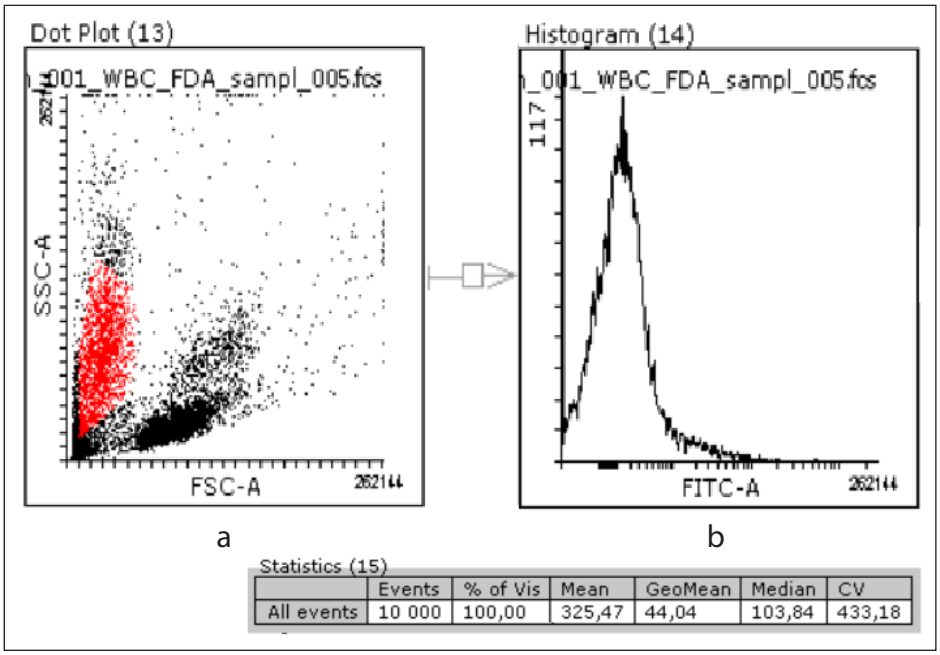


Fig. 1. Representative cytogram (a) and histogram SSC/FL1 (2,7 – dichlorofluorescein) (b) of leukocytes of rat No. 2 of group 1. Mean fluorescence intensity of DCF is 325.47.

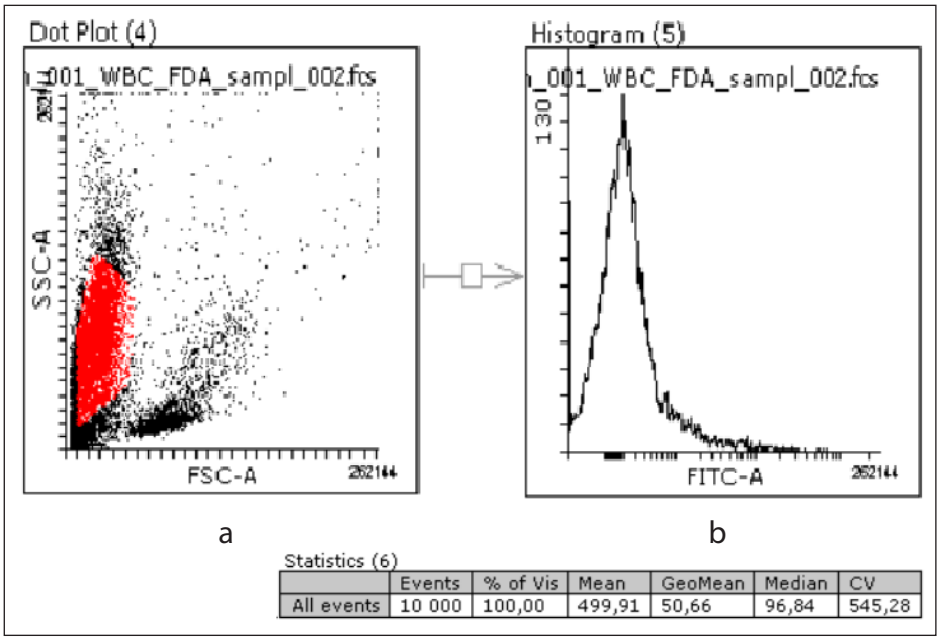


Fig. 2. Representative cytogram (a) and histogram SSC/FL1 (2,7 – dichlorofluorescein) (b) of leukocytes of rat No. 1 of group 2. Mean fluorescence intensity of DCF in leukocytes is 499.91.

sity of DCF in a population of leukocytes. The results obtained are presented in table I.

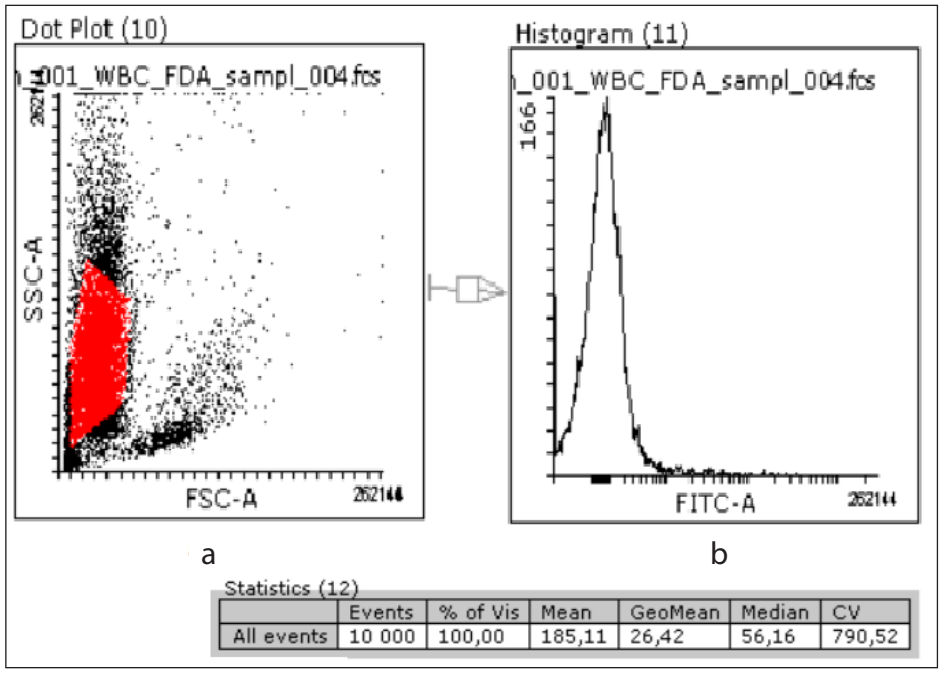
The MFI of DCF in group 1 coincides with the results obtained by us earlier [9] and will be used in this study as a standard indicator. Figure 1 shows the generation of ROS in leukocytes of rat No. 2 of group 1.

In group 2 the MFI of DCF in leukocytes did not significantly ($p>0.05$) differ from the indicator of group 1 (Table I). Figure 2 presents the results of evaluating the fluorescence intensity of DCF in leukocytes in rat No. 1 of group 2.

The MFI of DCF in leukocytes in group 3 was characterized by a tendency ($p>0.05$) to decrease compared to the indicator of group 1 (Table I). Figure 3 shows the results of the fluorescence intensity assessment of DCF in leukocytes in rat No. 4 of group 3.

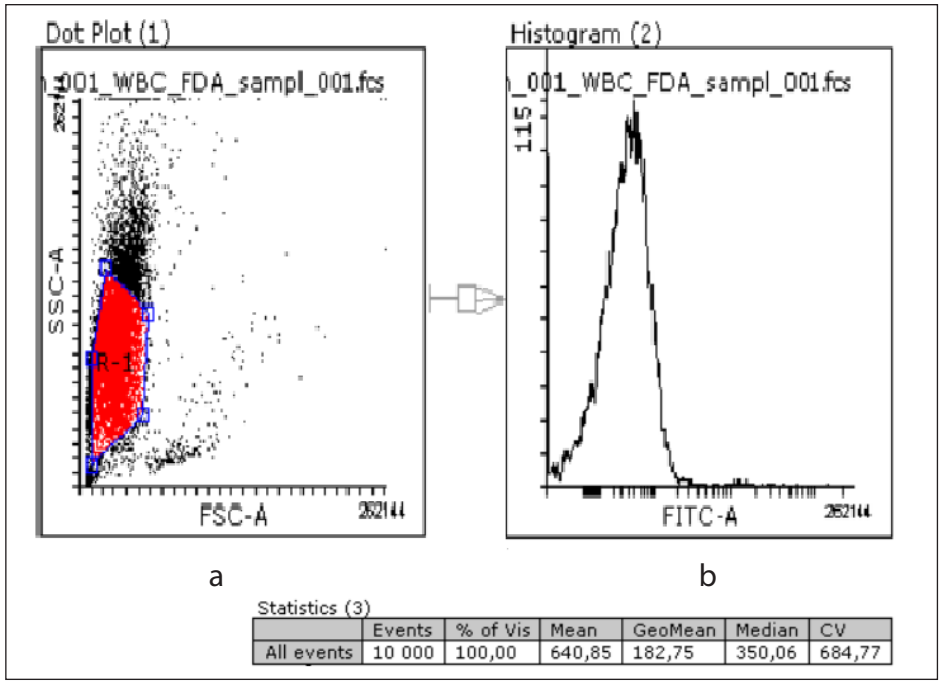
In group 4 the MFI of DCF was significantly ($p<0.05$) higher compared with the corresponding indicators of groups 1-3 (Table I). The results obtained in this group indicated the formation of a larger number of ROS compared to groups 1-3. Figure 4 shows the results of evaluating the fluorescence intensity of DCF in leukocytes in rat No. 3 of group 4.

In group 5 the MFI of DCF in leukocytes decreased ($p<0.05$) compared with the corresponding indicator of group 4 and did not significantly ($p>0.05$) differ from the corresponding indicators of groups 1-3 (Table I). Figure 5 presents the results of evaluating the



	Events	% of Vis	Mean	GeoMean	Median	CV
All events	10 000	100,00	185,11	26,42	56,16	790,52

Fig. 3. Representative cytogram (a) and histogram SSC/FL1 (2,7 – dichlorofluorescein) (b) of leukocytes of rat No. 4 of group 3. Mean fluorescence intensity of DCF in leukocytes is 185.11.



	Events	% of Vis	Mean	GeoMean	Median	CV
All events	10 000	100,00	640,85	182,75	350,06	684,77

Fig. 4. Representative cytogram (a) and histogram SSC/FL1 (2,7 – dichlorofluorescein) (b) of leukocytes of rat No. 3 of group 4. Mean fluorescence intensity of DCF in leukocytes is 640.85.

fluorescence intensity of DCF in leukocytes in rat No. 6 of group 5.

DISCUSSION

Blood leukocytes provide the body's immune status. The most numerous populations of leukocytes are neutrophils (47-72%), which are microphages and carry out phagocytosis of infectious agents. Morphologically, these cells have a segmented nucleus, an endoplasmic reticulum that does not contain ribosomes. There are few mitochondria in the cells, but they contain many different granules with various enzymes (peroxidases, hydrolases, alkaline phosphatase), proteins – lactoferrin, lysozyme, cationic proteins and others. The source of energy is glucose, which is oxidized in the pentose phosphate pathway, glycolysis reactions (90%) or can be transformed and stored as glycogen in tissues. An increase in the intensity of metabolic pathways for the conversion of glucose accompanies phagocytosis. During phagocytosis, the intensity of oxygen uptake by neutrophils increases with the formation of ROS: 1) superoxide anion formed with the help of

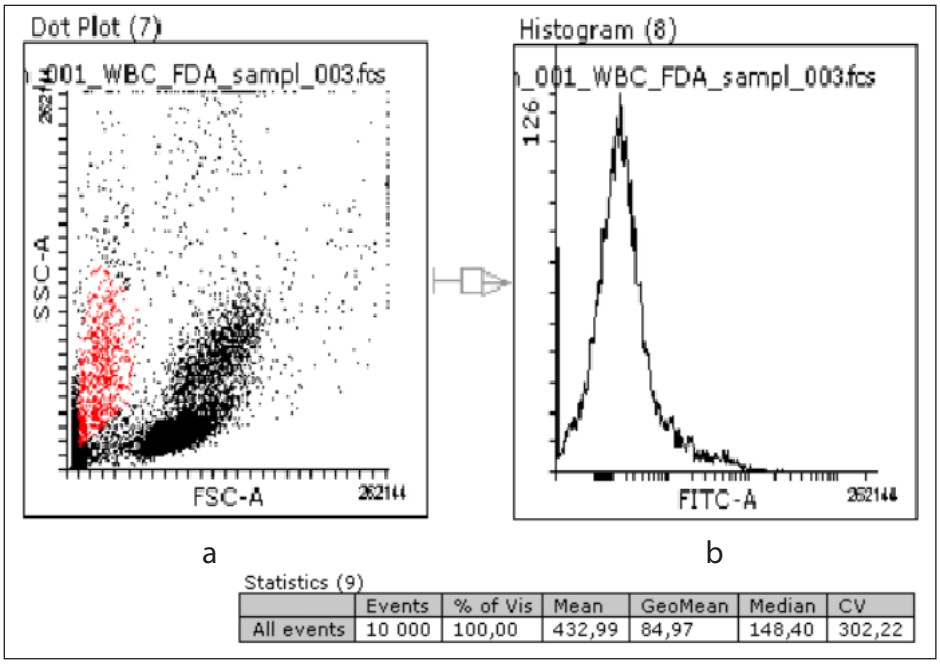


Fig. 5. Representative cytogram (a) and histogram SSC/FL1 (2,7 – dichlorofluorescein) (b) of leukocytes of rat No. 6 of group 5. Mean fluorescence intensity of DCF in leukocytes is 432.99.

oxidase (NADPH-dependent); 2) hydrogen peroxide, the formation of which depends on the activity of NADH-dependent oxidase; 3) hypochloric acid formed under the action of myeloperoxidase (hypochloric acid in the form of an anion reacts with hydrogen peroxide to form singlet oxygen); 4) free radicals, which have a powerful bactericidal effect, are unstable, active and interact with organic substances. Due to the formation of ROS in neutrophils, the bactericidal action of these cells is carried out, as well as the regulation of the functioning of the neutrophils themselves [9]. In the human body, the generation of ROS by neutrophilic leukocytes can have not only physiological or homeostatic, but also pathological significance [10].

ROS play a vital role in ovarian physiological activity as a secondary messenger for cellular signaling and are involved in the regulation of the ovarian cycle, including in meiosis, ovulation, corpus luteum maintenance, and regression [11].

Excessive generation of ROS and violation of the activity of antioxidant protection are the cause of oxidative stress. The latter, as is known, manifests itself as free-radical oxidation of lipids, proteins, and nucleic acids, i.e., initiates the lipid, protein, and nucleic mechanisms of cellular damage. Oxidative stress is the cause of the development of ovarian diseases including age-related ovarian dysfunction, ovarian cancer, PCOS, ovarian endometriosis [11, 12]. Overproduction of ROS may damage the oocytes and impair their fertilization capacity [13].

In this study, the author determined the excessive generation of ROS by leukocytes in experimental PCOS. Excessive production of ROS, from our point of view, is due to neutrophilic leukocytes, since these cells account for the largest percentage in the leukocyte formula. Excessive generation of ROS and the development of oxidative stress have also been noted in numerous studies by various scientists conducted on clinical and experimental material [14].

Cold exposure is one of the stressogenic factors. Literature data about the influence of cold on the processes of ROS generation and the occurrence of oxidative stress are debatable and contradictory. According to some scientists, cold exposure results in an elevation of metabolic rate in mammals, an imbalance in the antioxidant defense system, increased ROS production and oxidative stress. Elevation of ROS production correlated with an increase in energy generation [15]. The results of some studies indicate the activation of the antioxidant system (enzymatic and/or non-enzymatic) and a decrease in the production of ROS under conditions of cold exposure [16-18]. Contradictory data from the literature, from our point of view, are mainly caused by different characteristics of the cold factor (duration of action, degree of temperature reduction, etc.).

Our experimental study showed that the use of intermittent cold exposure (4 hours every day at a temperature of +4°C for 25 days) in rats with PCOS led to the normalization of the rate of ROS production by leukocytes. Taking into account the importance of hyperproduction of ROS in the etiopathogenesis of PCOS, the results allow us to recommend the intermittent cold exposure as one of the methods of prevention and treatment of the above ovary pathology.

In the literature of recent years, there are studies that use a technique similar to ours for modeling the PCOS and prove the effectiveness of using the cold exposure, aspirin, glutamine, vitamin D, vitamin C, marjoram, metformin, transplantation of brown adipose etc. as methods of treatment of this pathology [19-23]. In these studies, the effectiveness of the treatment methods was proven by studying various processes and mechanisms, including the oxidant and antioxidant systems.

CONCLUSIONS

The experimental study revealed an intracellular excessive production of reactive oxygen species by leukocytes in polycystic ovary syndrome. The use of intermittent cold exposure normalized the production of reactive oxygen species by leukocytes in rats with polycystic ovary syndrome. The effectiveness of intermittent cold exposure, proven by the authors, allows recommending its use as one of the methods of prevention and treatment of the polycystic ovary syndrome.

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ORIGINAL ARTICLE

ANALYSIS OF THE RESULTS OF A MEDICAL AND SOCIOLOGICAL SURVEY OF HEALTHCARE PROVIDERS ON MOTIVATIONAL COMPONENT OF ENSURING THE HEALTHCARE QUALITY

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ABSTRACT

The aim: To identify the main motivational components of improving the healthcare quality in health care providers in Sumy.

Materials and methods: The study involved data obtained from 187 doctors working in primary health care institutions, inpatient and outpatient departments of health care institutions in Sumy, during September-November 2020. The study used systematic, bibliosemantic approaches, comparative and statistical analysis, and logical generalization. The obtained data were processed and statistically analyzed with Google Forms and Microsoft Excel 2010 Windows.

Results: The analysis of data received from the surveyed group of respondents showed that 83 doctors (44.39%) have 11-15 years' work experience, 51 people (27.27%) – 6-10 years, 40 people (21.39%) have up to 5 years of work experience and 13 people (6.95%) – more than 15 years. Most doctors (114 people (60.96%)) work for one position, 39 people (20.86%) work for less than one, while 34 people (18.18%) work for more than one position. The number of doctors who indicated that they were mostly overworked during the working day was 123 (65.77%), another 46 (24.60%) indicated that they were overworked during the working day correspondingly to their workload at occupied position, and 18 respondents (9.63%) answered that they were not fully loaded. At the same time, 91.98% of people indicated that the actual amount of their salary does not correspond to the workload, and there is no financial stimulation system for medical care quality increasing (87.70% of responses).

Conclusions: The study showed that the healthcare facilities where the respondents work do not have an effective system of staff motivation for work quality (79.14% of responses). It was found that doctors are ready to work harder and better for additional pay, despite the high level of workload (88.24% of responses), and consider it necessary to introduce an effective stimulation system to improve the quality of medical services (96.79% of responses).

KEY WORDS: healthcare quality, health care provider, healthcare institution, healthcare quality management

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INTRODUCTION

The main issue of preserving and progressive development of health care today is ensuring the quality of the medical sector, which is related to the general aspects of the process of improving the management of professional activities of medical personnel, at state, regional and local levels. A major problem in many countries, including Ukraine, is low supply of healthcare workers, especially in rural areas, due to low motivation to work in the healthcare sector. The priority policy direction of many countries until 2030 involves development of human/medical resources for healthcare institutions. For example, China has proposed a partnership strategy for communities and the health care system (HCS), namely: joint ownership and program development, joint supervision and constructive feedback, a balanced package of incentives, both financial and non-financial, and a practical monitoring system that includes HCS data [1, 2].

The problem of improving the incentive system for healthcare providers is of relevance, as this approach can boost the work of healthcare professionals and increase their efficiency. Today, in most municipal and even private healthcare institutions, healthcare providers are paid based on a single salary scale, including all additional payments. This type of remuneration is fixed and therefore does not depend on the quality and content of medical services provided, so healthcare providers receive a fixed salary even if they have not completed a certain amount of work. This situation indicates that the remuneration system fails to fulfill one of the most important functions of stimulating highly efficient work and improving the quality of medical care (QMC) [3, 4].

Methods, techniques, and tools of motivating have undergone a long evolutionary way. For many years, the model of physical coercion to work was dominant, followed by the models of economic necessity and

incentives based on productivity. Motivation of health care staff is closely related to the process of encouraging professionals to work through the formation of behavioral motives to achieve personal goals and goals of health care institution, as motivation process involves the use of interdependent categories in a certain sequence: needs of health care workers – their interests – motives for professional activity – actions [5, 6].

Today, it is necessary to improve the mechanism of incentives and psychosocial environment for healthcare providers in healthcare institutions to ensure they perform their professional duties with high quality. From the management level there should be moves to provide support and financial incentives to healthcare workers due to the constant increase in their workload, high level of responsibility, difficulties in the work process, and presence of stressful professional factors, which leads to emotional, mental, and nervous tension, "burnout", and risk of losing health while performing their professional duties. The experience of management in medical field shows that the traditional methods of the system for assessing the motivational orientation of healthcare providers no longer meet the needs of the management apparatus [7, 8].

The relevance of developing a high-quality system of healthcare providers` motivation in healthcare institutions contributes to identification of effective tools and incentives to impact the behavior of healthcare providers to achieve their own goals and the goals of the healthcare facility [9]. However, despite many studies in this area, the problem of healthcare providers' motivation has not been solved in Ukraine. Despite the obvious economic, social, and organizational feasibility, the problem of motivation remains a priority for the state to overcome in the healthcare sector.

THE AIM

To identify the main motivational components of improving the healthcare quality in health care providers in Sumy.

MATERIALS AND METHODS

The study involved doctors working in primary health care institutions (PHIs), inpatient and outpatient units of health care institutions in Sumy, during September-November 2020. A total of 187 respondents took part in the survey. The questionnaires were reviewed and approved by the Academic Council of the Educational and Research Medical Institute of Sumy State University. The study used a systematic and bibliosemantic approaches, comparative and statistical analysis, and logical generalization. Using the functions of Google Forms and Microsoft Excel 2010 for Windows, the data were processed and statistically analyzed.

RESULTS AND DISCUSSION

The healthcare providers who participated in the survey were divided into professional groups, depending on their place of work: specialized doctors, who work in outpatient departments – 73 people (39.04%), specialist doctors, working in inpatient departments of therapeutic profile – 54 people (28.88%), general practitioners-family doctors (GP-FD) – 45 people (24.06%), specialist doctors working in inpatient departments of surgical profile – 15 people (8.02%) (Table I).

When distributing the respondents by work experience in healthcare system, it was found that 83 people (44.39%) have 11-15 years` work experience, 51 people (27.27%) – 6-10 years, 40 people (21.39%) – up to 5 years, and 13 people (6.95%) – more than 15 years.

Most of the surveyed doctors (114 people (60.96%)) work at on position, 39 people (20.86%) work for less than one position, 34 people (18.18%) work for more than one.

As per the survey results, most doctors indicated that they were overworked during the working day (123 people (65.77%)); another 46 people (24.60%) indicated that they were loaded correspondently with workload of their position during the working day, and 18 respondents (9.63%) said that they were not fully occupied. It should be noted that all doctors who indicated that their workload was less than the full-time, work in inpatient departments of HCFs. Among the doctors who, in their opinion, are overworked, the vast majority work in outpatient departments – 68 people (55.28%) and in primary healthcare institutions – 44 people (35.77%). Only 11 people (8.94%) working in inpatient departments reported being overworked. These data confirm that many healthcare services are provided in outpatient settings, particularly at the level of primary care centers, which requires more material, technical and human resources.

The survey showed that 56.68% of respondents are satisfied with material and technical support and working conditions at the workplace; at the same time the share of respondents who were dissatisfied with existing working conditions equaled 43.32%. At the same time, healthcare providers who do not have a certification category or have the second category are less satisfied with their working conditions than healthcare providers with first or higher certification category.

Almost all doctors (91.98% of responses) indicated that the actual amount of their salary does not correspond to the workload, and only 8.02% of the surveyed doctors are satisfied with their salary.

Analyzing the answers to the question "Do you receive a surcharge for increasing of quality of medical service?", it can be stated that there is almost no system of financial incentives for doctors for quality medical care: 164 people

Table I. Distribution of respondents by professional groups and place of work (in absolute numbers and %)

No	Group of doctors by professional distribution	Number of respondents in absolute value (persons)	Number of respondents in relative value (%)
1	2	3	4
1	General practitioners-family doctors (GP)	45	24.06%
2	Physicians who work in the polyclinic departments	73	39.04%
3	Physicians who work in the therapeutic inpatient departments	54	28.88%
4	Physicians who work in the surgical inpatient departments	15	8.02%
5	In total	187	100%

(87.70%) gave a negative answer, only 23 people (12.30%) said that they received a surcharge for high quality work. All surveyed healthcare providers with less than 5 years of work experience did not receive any additional payments for quality of work at all to be mentioned.

Despite the high level of doctors' workload, most of respondents (165 people (88.24%) answered the question "Are you willing to work more for an additional payment?" positively. Only 22 people (11.76%) said they did not want to perform extra work for payment.

The study identified the motivating factors of respondents that made them choose a doctor carrier (the question with multiple choice). The answers received show that more than half of the respondents (57.75%) chose their profession because they want to benefit and help people; 41.18% of respondents said they work as doctors because of their professional interest; 31.55% of people – to feel needed by people; 25.13% of people – to help treat their family and friends; 29.95% of people – to obtain material benefits; 24.6% of people mentioned social status, stable job, and public respect for the profession as motivating factors.

Despite the low level of material satisfaction in respondents, the majority (139 people (74.33%)) are not ready to change their profession to another with higher salary; 48 people (25.67%) would like to change their profession to receive higher payment. Respondents with more years of working experience (11 years and more) are more likely to be neither ready nor willing to change their profession (19.79%) than respondents with less than 10 years of experience (50.80%).

To the question "In your opinion, does the healthcare institution where you work have a system of motivation for medical staff?" 148 people (79.14%) answered negatively, only 19 people (10.16%) confirmed the existence of a motivation system, and another 20 people (10.70%) indicated that this system exists partially.

According to healthcare providers, the main incentives to improve QHC are (multiple answers were possible): financial (73.26%), respect of patients and society in general (51.87% of answers), well-established teamwork and moral satisfaction from work (47.05%), opportunities for professional and career growth (35.83%), and recognition and respect from management (6.42% of answers).

The survey demonstrates that almost all doctors (181 people (96.79%)) believe that effective mechanisms for motivating the provision of QHC should be introduced.

The data obtained during our study proves that healthcare providers are positive for implementation of an effective incentive system to improve QHC, yet all they agree that at present moment the motivational component is practically absent in Sumy healthcare institutions. It has been established that regardless of work experience and place of work, one of the main motivational factors for health care providers is moral satisfaction from work and gaining respect from society. Thus, despite the low level of salaries, the main priorities for medical staff have been and remain the principles of humanism and ethics.

CONCLUSIONS

1. The survey of healthcare providers showed that the vast majority (164 people (87.70%)) does not receive additional payments for quality work. In addition, 79.14% of respondents noted that the hospitals where they work do not have effective system of staff motivation for the quality of medical care.
2. It was found that a large scale of doctors (139 people (74.33%)) are not ready to change their profession to another with higher payment, even given a low level of their material satisfaction.
3. It was found that the respondents are ready to work more and better for additional pay, despite the high level of workload, namely 165 people (88.24%) are ready for this. In addition to material incentives, doctors mentioned moral satisfaction from work (47.05% of responses) and respect from patients and society as a whole (51.87% of responses) as important motivational factors for improving the quality of care.
4. The survey has shown that almost all healthcare providers (181 people (96.79%)) consider it necessary to introduce an effective system of motivation at the level of healthcare institutions to improve the quality of care.

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REVIEW ARTICLE

THE PROBLEMS OF CRIMINAL LIABILITY OF PHARMACEUTICAL EMPLOYEES IN THE CONTEXT OF CERTAIN FORMS OF COLLABORATIVE ACTIVITIES

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ABSTRACT

The aim: Based on the specifics of the criminal legislation, which provides liability for collaborative activity, it is necessary to offer an adequate understanding of the limits of the criminal liability of pharmaceutical employees for these criminal offenses, as well as to determine the characteristics of the components of these criminal offenses related to the pharmaceutical activities carried out by pharmaceutical employees.

Materials and methods: The conducted research is based on the analysis of the provisions of the criminal legislation of Ukraine and Georgia.

Conclusions: The main problems of criminal liability for collaboration activities by pharmaceutical employees are connected to the following: without clarification of the aforementioned criminal offenses, it is impossible to specify the limits of criminal liability.

KEY WORDS: criminal liability, collaborative activity, pharmaceutical employers, criminal offence

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INTRODUCTION

Since the current criminal legislation of Ukraine has newly introduced concept of "collaborative activities", there are problems of criminal liability of certain categories of persons (employees) performing professional functions, as well as the selection of the types of actions provided in the current Criminal Code (hereinafter referred to as the CC) of Ukraine and under certain conditions may constitute criminal offenses connected with collaborative activities.

The lack of ways to solve these problems hinders the efficiency to implement mechanisms for prevention criminal offenses typical to the criminal legislation connected with assistance of certain categories of persons (employees) performing professional functions in collaborative activities carried out by them in certain forms.

Thus, with the start of a full-scale military invasion in February 2022, CC of Ukraine was amended with new article, which is criminalizing any type of the cooperation with an aggressor state. These new mechanisms, although "included" at the normative level, vague and broad definition of collaborationism and types of collaborative activities can become problematic for law enforcement agencies and judicial bodies.

Provisions regarding "collaborative activities" are not widely used in criminal legislation. Thus, the experience of Georgia, which has art. 322-2 in CC of Georgia [1] and special Law on Occupied Territories [2] could be an example that can be used for comparison.

THE AIM

The aim of this study is to propose an adequate understanding of the limits of the criminal liability of pharmaceutical employees for collaborative activities. Also, to determine which of the specific types of criminal offenses provided by criminal legislation, so called "fit" the content of such a "subject" of collaborative activities of pharmaceutical employees.

MATERIALS AND METHODS

The conducted research is based on the analysis of the provisions of the criminal legislation of Ukraine and Georgia. We compared provisions of criminal legislation and formulate proposal for improving certain provisions of Ukrainian criminal legislation, which provide criminal liability for assistance in committing subversive activities against state and collaborative activities carried out by certain categories of employees performing professional functions (focusing on pharmaceutical employees).

REVIEW AND DISCUSSION

1. CRIMINAL LEGISLATION OF GEORGIA

In 2008, after the Russian-Georgian war of August 2008, CC of Georgia was amended with Chapter XXXVII "Violation of the legal regime of the occupied territories" which contains from 2 articles. Also, special Law of Georgia on the Occupied Territories had been passed. Article 322-2 of CC of Georgia defined that crime is conducting "… economic activities in the occupied territories that are prohibited under the Law of Georgia on the Occupied Territories". [1] Also, for the act specified in this article, a legal person could be punished by fine, deprivation of the right to carry a particular activity or by liquidation.

According to the Law of Georgia on Occupied Territories, any economic activity in Abkhazia and South. Ossetia without prior authorization from Georgian authorities are violation of legislation provision. Mentioned legal act prohibit any economic activity in the occupied territory which, "…irrespective of whether it is carried out to gain profit, income or compensation, if an appropriate licence or a permit, authorization or registration is required to conduct this activity under the Laws of Georgia on Licences and Permits,…" [2]. Obviously, the activities of pharmaceutical workers fall under the provisions of this law (making a profit and the need to obtain an appropriate license).

However, economic activity in these territories has not been much of an issue, as there is simply little economic activity there, [3] and what is more, ties and business contacts with the rest of Georgia territory are limited. As for 2017, there were only 4 cases of illegal economic activity in occupied territories. [4] Despite the direct prohibition of any business activity in Georgian occupied territories, the effectiveness of the implementation of mechanisms for bringing to criminal responsibility remains at an insignificant level.

2. CRIMINAL LEGISLATION OF UKRAINE

Since temporary occupation of Crimea and part of Ukraine's territory in the east, special law of Ukraine "On Ensuring Civil Rights and Freedoms, and the Legal Regime on the Temporarily Occupied Territory of Ukraine" [5] had been passed, where art.13 had been regulating peculiarities of economic activity in the temporarily occupied territory.

Thus, with the start of a full-scale military invasion in February 2022, CC of Ukraine was amended with number of crimes regarding criminal liability for cooperation with the aggressor in, inter alia, economic spheres. Under the new regulation, collaborative activities can take various forms. Among them: conducting economic activities in cooperation with the aggressor state and illegal authorities.

The composition of this crime (part 4 article 111-1 CC of Ukraine) is formulated in the following way:

"Transfer of material resources to illegal armed or paramilitary formations created in the temporarily occupied territory and/or armed or paramilitary formations of the aggressor state, and/or conducting economic activities in cooperation with the aggressor state, illegal authorities created in the temporarily occupied territory, including the occupation administration of the aggressor state". [6]

Thus, it is prohibited for all businesses registered in Ukraine to carry out any economic activity in cooperation with the occupation administration. Based on the objectives of our article, the question arises – could pharmaceutical workers be punished, if they, for example, continue their business in occupied territories?

3. DETERMINING OF COLLABORATIVE ACTIVITIES WHICH CAN BE COMMITTED BY PHARMACEUTICAL EMPLOYEES

Clarification of the main forms of collaborative activity which in part 1-7 article 111-1 of the CC of Ukraine acquire the meaning of certain types of criminal offenses with independent compositions in the CC and other acts of criminal legislation is absent. Therefore, the following should be taken into account when clarifying the main forms of collaborative activity that can be committed by pharmaceutical employees:

3.1. Definition of "pharmaceutical employees" in the context of "collaborative activities". Pharmaceutical employees are a special subject of crime. [7] Basically, these are pharmaceutical employees engaged in pharmaceutical activities, who have the appropriate special education and meet the uniform qualification requirements. Also, pharmaceutical employees, as special subjects of the crime, must hold the relevant position of a pharmacist, a specialist with a pharmaceutical education, in accordance with the list established by pharmaceutical legislation. Provision of parts 1-3, 5, 7 Article 111-1 of the CC of Ukraine provides liability for "citizens of Ukraine", although foreigners or stateless persons may commit acts of assistance in committing subversive activities against Ukraine or any form of collaborative activity.

3.2. Limitation of the range of subjects to only citizens of Ukraine. This means that the issue of the importance of persons without citizenship or with "dual" citizenship must be resolved (this is a matter of administrative legislation, but from the point of view of criminal

law assessment it is important that foreigners and persons without citizenship under certain conditions can be accomplices in collaborative activities). After all, pharmaceutical employees can be all the specified categories of persons.

3.3. It is important to pay attention to the fact that economic activity is carried out by a business entity. In the case of establishing the facts of the carrying out of such economic activity, it is necessary to determine who acted and made decisions on behalf of such a business entity. This will be, first of all, a manager (director), a person performing his duties, or another person who acted on behalf of a business entity (during negotiations, conclusion and signing of a contract, delivery of goods, works, services). Persons who performed technical functions (storekeepers, pharmacists), depending on their intention, may be accomplices in such a crime, or their actions may not fall under the signs of the objective side of the crime (performance of economic activity). [8]

3.4. Medicinal products, with which pharmaceutical activity is carried out, can be material resources within the meaning of part 4 Art. 111-1 of the CC of Ukraine, which are transferred by pharmaceutical employees to "illegal armed or paramilitary formations created in the temporarily occupied territory, and/or armed or paramilitary formations of the aggressor state". However, the concept of "material resources" is not defined in the CC of Ukraine and non-criminal legislation. When defining them at the theoretical-and-applied level, it should be taken into account that these are any stocks in tangible and stuff form that can be used for the purpose of their further use or consumption (ie, including medicinal products).

3.5. Pharmaceutical activity performed by pharmaceutical employees is a type of economic activity. But what concrete forms of such economic activity could be classified as performed with cooperation with the aggressor state, illegal authorities created in the temporarily occupied territory". In fact, any supply of goods, performance of works, service provision could be. So, do the act of selling a medicine to a customer constitutes crime? Or paying taxes on an account of illegal authorities? Thus, pharmaceutical activity should be defined. It is obvious that pharmaceutical activity will have certain limits, which, in our opinion, will not intersect with any form of collaboration activity. One of the possible options is to supplement Law "On Medicines" with the provision that "pharmaceutical activity – activities related to the creation, pharmaceutical development, preclinical research, clinical research (trials), state registration of medicines, production, manufacture (production) in the pharmacy, appointment, application, import, wholesale and retail trade, distance trade, quality control of medicines, implementation of pharmacovigilance. (the preamble of the Law of Ukraine "On Medicines" dated July 22, 2022 [9] is a normative reference for such a definition of the concept of pharmaceutical activity). Further, it is the pharmaceutical activity that cannot be carried out without public authorities (strictly – illegal) that should constitute a crime.

3.6. Under certain conditions, a pharmaceutical employee may hold a position and perform the functions specified in part 5 Art. 111-1 of the CC of Ukraine. However, in this case, it is necessary for criminal liability to establish not only holding by a pharmaceutical employee a certain position (that he/she is in a certain way enrolled in a personnel unit), but first of all his/her performing the corresponding specified functions. These functions belong to the sphere of public law (they have the nature of powers of authority, and their normative reference point is the provision of part 1 of the note to Art. 364 of the CC). Therefore, the presence of a pharmaceutical employee in a position in the field of private law and the performance of certain functions (powers) by him/her can be taken into account when characterizing them as accomplices in collaborative activities.

CONCLUSIONS

When solving the main problems of criminal liability for collaboration activities by pharmaceutical employees, the following should be taken into account: possible problems are connected with the legislative explanation (concretization) of the forms of collaborative activity, distinguished by the legislator in parts 4 and 5 article 111-1 of the CC of Ukraine and acquire the meaning of certain types of criminal offenses with independent compositions.

The main problem of part 4 article 111-1 of the CC of Ukraine affecting criminal liability of pharmaceutical employees is the lack of legislative explanation of the concept of material resources (in particular, whether medicinal products as a subject of pharmaceutical activity performed by pharmaceutical employees may recognize as material resources). The concept of material resources is absent in the current legislation, and when determining them, it is necessary to take into account tangible and stuff materials able to be used for the purpose of their further use or consumption (including medicinal products in the process of performed pharmaceutical activity).

Since pharmaceutical activity performed by pharmaceutical employees is a kind of economic activity, then the current legislation should formulate the definition of the concept of pharmaceutical activity.

In addition, organizational-and-administrative and administrative-and-economic functions in the field of public law and their powers in the activities of legal entities in private law should be specified in the legislation on pharmaceutical activity. This legislative specification directly influences the solving of the main problems of criminal liability of pharmaceutical employees for the form of collaboration activity.

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REVIEW ARTICLE

LEGAL REGULATION OF THE INSTITUTE OF TRANSPLANTATION IN UKRAINE

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ABSTRACT

The aim: The aim of the study is to generalize the established by scientists features of the legal regulation of the institute of transplantation in Ukraine and other countries.

Materials and methods: The article examines the institute of transplantation, its medico-legal character, and the problems of implementing the institute in Ukraine. In the study, the authors applied general scientific methods, which include system analysis, system modeling, dialectical method. The authors used the following materials: laws, decrees of the President of Ukraine, resolutions of the government and ministries of healthcare, dissertations and articles by scientists, assessments of leading experts in the field.

Conclusions: Theoretically, the legal aspect of the study of the institute of transplantation is important for formulating the general patterns of its emergence, developing prospects for its functioning and strategic directions for its further development, building a system for protecting the rights of all participants in this legal relationship. Different aspects of transplantation can be considered separately: medical or surgical; biological; psychological. But there is an equally important aspect – the legal one, which reveals the institute of transplantation from the standpoint of the protection of human dignity.

KEY WORDS: transplantation, biological material, implant, medical service, donor

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INTRODUCTION

It can be said without a doubt that the perfection of legislation that regulates any sphere of activity is an important condition for the effective performance by the state of its functions. Activities in the field of health care, medicine and in such a significant area as the transplantation of human anatomical materials are no exception. After all, the life and health of every member of society is recognized as the highest value, which determines the relevance of the chosen topic.

Transplantology poses a number of moral and ethical, legal and religious questions to society, the answers to which may be contradictory from the point of view of morality, religion and law. However, the existence of real legal relations in the field of transplantation gives rise to the need to find compromises between the specified systems of social rules. That is why the issue of regulation of this activity at the legislative level is important. Practice proves that, for the most part, the lack of clear rules and responsibility in the field of transplantation gives rise to illegal trade in organs or tissues, forced donation and even killing for the purpose of extracting organs or tissues from a person. All these negative factors and possible consequences encourage us to regulate medical and legal activities related to transplantation within the limits of the law.

If a person's health is at risk and/or he or she is disturbed by thoughts of a fatal disease, it is difficult to focus on anything else, to think about economic benefits, procreation, personal development, career growth. Staying alive and being healthy becomes a person's priority above all listed above [1]. The quality of life of the population significantly depends on the level of development of medicine, in particular, such a field as transplantology. The need for donor organs grows every year, which requires the development of an effective mechanism for regulating legal relations in this area [2].

Today all over the world transplantation is considered an extremely effective and generally non-alternative method of treatment for diseases of vital organs, including heart, kidneys, liver, lungs, and others. That is why transplantation in developed countries is one of the most dynamically developing areas of medicine. The problem of legal regulation of human organ transplantation is being solved to a greater or lesser extent by Ukrainian and foreign scientists: H. Anikina [3], O. Gel [4], S. Grinchak [5], V. Shulga [6], D. Zadykhailo, V.

Milash, V. Yarotskyi [7], O. Shevchuk [8, 9], V. Trofymenko, I. Martynivskyi, G. Honcharenko, D. Zatinatskyi [8], O. Lysoded, I. Borysenko, O. Bababeva [9] and others.

The flourishing of successful transplantation practices in the developed countries of the world at the end of the 20th and the beginning of the 21st centuries and pronounced lag in it in Ukraine lead to an urgent need to investigate the problem. The sphere of health care is one of the most important areas that require legal regulation and control, based on the fact that human life and health are the highest social value [10]. In addition, transplantation concerns two people at the same time – the donor and the recipient, and in the opinion of O. Stets, O. Bylochenko and Yu. Chabanenko, medical personnel as well, which brings it closest to legal science and requires the "clearer" legal regulation among all medical disciplines" [11, 8]. The extreme importance of the problem is also confirmed by the decisions of various international organizations, which define the basic principles of transplantology, which are recommended to all states [12]. In the context of integration into the European Union, Ukraine undertook to make efforts to harmonize its legislation with the legislation of the European Union, including in matters of human organs transplantation [13]. The national legislation on transplantation of human organs consists of a large number of normative legal acts, which leads to the problem of inconsistency of some norms and the need to make the necessary changes to the current legislation.

THE AIM

The aim of the study is to generalize the established by scientists features of the legal regulation of the institute of transplantation in Ukraine and other countries. The materials of the article are valuable for and can be used by transplantologists, students of medical universities specializing in this area, medical law teachers of legal educational institutions, students of the relevant profile and all those who are interested in the raised issues.

MATERIALS AND METHODS

The article examines the institution of transplantation, its medico-legal character and legal regulation, the problems of implementing the institution, the mechanisms of its implementation in a modern democratic society. The methodological basis of the research is general scientific methods, which include system analysis, system modeling, and the dialectical method.

In order to determine the characteristic features of the laws of Ukraine on the transplantation of human organs, the authors used the following materials: laws, decrees of the President of Ukraine, resolutions of the government and ministries of healthcare, dissertations and articles of scientists, assessments of leading experts in the field, as well as theoretical scientific methods and techniques of research. The comparative-legal method contributed to the generalization of the experience of developed countries in the field of transplantation. The historical-legal method was used in the study of the formation and development of the administrative-legal regulation of the institute of transplantation in Ukraine. The formal-legal method provided an analysis of the powers of subjects of state management of transplantation in Ukraine. The technical-legal method was used to interpret the provisions of law of the institute of transplantation. The method of legal forecasting was used to determine a complex of possible options for the development of transplantology in Ukraine.

REVIEW AND DISCUSSION

Despite the ongoing war on the territory of our country and the current special legal regime [14], scientists of the country are trying to pay due attention to the vital issues of the development of medical and legal institutes in Ukraine, and the institute of transplantology is no exception. Transplantation is a specialized method of treatment, which is that the organs or cells from one person (donor) are transplanted to another (recipient). Today, scientists predict that in 30 years, 60% of surgical interventions will be related to transplantation. These forecasts are based on the global rate of growth in the number of transplant operations. There is no country on the planet in which the institution of transplantation is prohibited. The World Health Organization notes that 104 countries of the world have the financial, material, technical and human resources to carry out the transplantation of organs or cells from the donor to the recipient [15]. P. Humel and his colleagues, conducting an assessment of the functions of national ethics committees around the world, systematized bioethical topics discussed on websites and contents of well-known authors. The search was conducted in English, French and Spanish. Discussion topics were grouped into thematic categories through an iterative regrouping process to come to a core set of topics. The five main topics of these publications were: research ethics (124; 9%), genetics and genomics (62; 6%), organ transplantation (58; 5%), assisted reproductive technologies (49; 4%) and end of life (36; 3%) [16]. We see that the topic of organ transplantation is a relevant and widely discussed topic in scientific doctrine.

More than 24,000 organ or cell transplant operations are performed annually in the USA, more than 4,000 are performed in Spain, and more than 1,500 transplantations are performed in Poland [17, p. 66]. This branch of medicine is actively developing in the Baltic countries, in particular, in Estonia, where the rate of organ and cell transplantation per 1 million population is 46.2, the rate in Latvia – 36.2, in Lithuania – 22 [15].

The era of modern organ transplantation was "historically opened" by our compatriot Professor Yu.Yu. Voronim (1895-1962) [18], who in 1933 performed the world's first cadaveric kidney transplant to a person in the city of Kharkiv (where he was undergoing an internship). It was the first attempt in history to transplant a whole organ into a person. An elderly man with a severe brain injury was brought to the clinic; he died peacefully in the emergency room. On the same day, a young woman who attempted suicide was dying in the clinic: she had taken a fatal dose of sulema. Unfortunately, the girl was hospitalized only four days after the suicide attempt. Her body was sick for four days, the kidneys were not functioning. The surgeon performed extirpation of the right kidney of the corpse, transferred it to the operating room to the patient. After applying vascular sutures, the blood circulation in the kidney was restored, the blood supply was sufficient. The kidney was included in the blood circulation and began to function on its own. The first recipient lived with the new organ for only 48 hours [19].

Today, the annual need for organ transplantation in the country is 3,653 cases, of which 2,115 are kidneys, 830 are liver, 30 are pancreas, 89 are pancreas and kidney together, 328 are heart, 240 are lungs, 3 are heart and lungs together, intestines are 42 cases. At the same time, transplant operations part is 0.8% of all surgical interventions. According to the Ministry of Health, 5 liver transplantations, 2 kidney transplantations, and 93 complex transplantations were performed in Ukraine in 2016 [20].

The material base for this extremely important branch of medicine was developing in Ukraine. Six centers received the right to perform operations on the transplantation of human organs: the National Institute of Surgery and Transplantation named after O. O. Shalimov, Lviv Regional Clinical Hospital, Odesa Regional Clinical Hospital, Regional Clinical Center of Urology and Nephrology named after V. I. Shapoval (Kharkiv), Zaporizhzhia Regional Clinical Hospital, Dnipropetrovsk Regional Clinical Hospital named after I. I. Mechnikov, which are able to carry out up to 1,000 organ transplantations per year [21].

The legislative framework for the implementation of human organ transplantation was also developing. The basis of the regulation of this legal institution is laid down in the norms of the Constitution of Ukraine [22] and the Civil Code of Ukraine [23]. For a more detailed clarification of the mechanism of regulation of the organ and cell transplantation institute, we should refer to the Law of Ukraine "On the Application of Transplantation of Anatomical Materials to Humans" [24], Fundamentals of the Legislation of Ukraine on Health Care [25], which, in turn, play a role of the medical activity code. In order to quickly regulate medical activities in the field of transplantation, by-laws of executive authorities are adopted, including, for example, the order "On establishing diagnostic criteria for brain death and procedures for determining the moment of death of a person" [26].

The adoption of the Law of Ukraine "On the Application of Transplantation of Anatomical Materials to Humans" [24] became the basis for the formation of the newest stage of transplantology in Ukraine. Changes in legislation in the field of organ and cell transplantation allowed:

– to improve the terminological apparatus of this institute;
– to make changes to the criminal legislation regarding increased liability for illegal removal and transplantation of anatomical materials to a person (Article 143 of the Criminal Code of Ukraine [27];
– to define the concept of "the moment of irreversible death" by making appropriate changes to the basic norms of health care. The "moment of irreversible death" is proposed to mean the biological death of a person or the moment of the death of his or her brain. In practice, the ascertaining of death is confirmed by a council of doctors and the drawing up of a legal act signed by all council participants. The specified legal document is kept in the patient's medical records.

In international law, issues regarding the institution of transplantation are regulated by the Helsinki Declaration of the World Medical Association "Ethical Principles of Medical Research Involving Human Subjects" from 1964 [28], which states that progress in medicine is based on works that ultimately include research with the participation of a human as an object of research. The purpose of such research is the need to explain the causes and consequences of diseases, to improve existing preventive, diagnostic, and therapeutic measures. The following international document establishing the principles of legal regulation of the institution of transplantology is the International Declaration on Transplantation of Human Organs of 1987 [29], which

declares that both the donor and the recipient are patients, and therefore all measures must be taken to protect the rights of both. Finally, the 1997 Convention on the Protection of Human Rights and Dignity of the Human Being with regard of the Application of Biology and Medicine [30] and its Protocols emphasize that modern biology and medicine are developing at an accelerated pace, and their improper use can lead to actions that threaten human dignity. Therefore, progress in these fields should be used for the benefit of present and future generations, while the interests of an individual should be higher than the interests of the entire society or science.

Systematizing the problems of the institute of transplantation, three main aspects should be highlighted.

First, the Law of Ukraine "On the Application of Transplantation of Anatomic Materials to Humans" [24] establishes the mandatory consent of relatives (a spouse, children, parents, brothers, sisters) for the removal of biological materials from the human body for transplantation and the production of implants. In the absence of relatives, consent for transplantation can be given by a person who has the duty to carry out a person's burial. In the event of the death of a person under the age of 18, consent to the selection of materials is given by the child's parents or the legal representatives. As a rule, such consent is not given in practice. And in cases where consent is given, the need to prepare a large number of legal documents plays a negative role as a psychological and emotional factor and affects decision-making.

When obtaining consent for donation, a significant role is assigned to the coordinator, who organizes communication with the persons giving consent for the removal of organs and cells from the donor. The coordinator first contacts the persons who can give consent to transplantation by phone, finds out the degree of their family connection with the donor, their place of residence, and then organizes a personal meeting. During the meeting, the coordinator informs the authorized person about the provisions of the current legislation of Ukraine, explains the goals of removing the organs or cells of a deceased person, informs about the voluntariness of consent to the donation of anatomical materials that must be taken with awareness of the meaning of the actions, without any coercion. During the meeting with the coordinator, other close relatives and family members or other legal representatives of the deceased may be present. If it is necessary to provide additional explanations regarding donation, brain death, biological death, the coordinator can involve doctors of other specialties in such explanations.

Obtaining consent from the relatives of the deceased is of great importance for the observance of the rights of all participants in legal relations concerning organ transplantation. This thesis is revealed, in particular, in the decisions of the European Court of Human Rights. And although the Convention on the Protection of Human Rights and Fundamental Freedoms does not directly provide for the protection of the right to health, applying for the protection of this right is possible on the basis of Articles 2, 3, 5, 8 of the Convention. Thus, in the case "Petrova v. Republic of Latvia" [35] the applicant pointed to the fact that after the death of her son as a result of a traffic accident, his kidneys and spleen were removed for the purpose of organ transplantation without her consent or her son's prior consent to these actions. The applicant became aware of what had happened nine months later during the criminal proceedings regarding the traffic accident in which her son had died. The country's authorities emphasized that such actions corresponded to the current Latvian legislation, since at the time of the applicant's son's death, the provisions of the law were in force, which did not oblige medical professionals to search for and inform relatives of the deceased about the possible removal of organs, as well as clarify their right to object against the removal of his organs or tissues. The European Court of Human Rights emphasized that Latvian legislation was not formulated clearly enough and did not provide effective protection against arbitrariness. A similar situation is reflected in the case "Elberte v. Republic of Latvia" [36], which concerned the removal of the body tissues from the applicant's deceased husband and their transfer to a pharmaceutical company in Germany for medical experts without her knowledge and consent. Elberte became aware of this situation only two years later, when a criminal investigation was launched in the Republic of Latvia in connection with the assumption of large-scale illegal removal of organs and tissues from the dead.

Here we should mention the above Convention on the Protection of Human Rights and Dignity of Human Being with regard to the Application of Biology and Medicine. This Convention is the completion of the codification of the principles of bioethics and the starting point for the development of the transplant institution in the future. Currently, 35 countries have signed the Convention, 29 of them have ratified it. The main achievement of it is that it became the first legally binding international instrument that covered a huge range of ethical issues in the field of biological research, indicated a wide range of ethical and legal principles applicable to medical actions and new

biomedical technologies. Article 29 of the Convention provides for the right of the European Court of Human Rights to formulate conclusions on legal issues regarding the interpretation of the text of the Convention. Many principles of the Convention are directly related to private law and categorical requirement to obtain consent for medical intervention. The Convention can be called a treaty on patient rights, which acts as a codification of the principles of medical practice. The focus of the norms of the specified international document is expressed in the protection of the dignity and individual integrity of a person, guaranteeing the observance of the inviolability of the person in connection with the application of the achievements of biology and medicine. The Convention introduces four key principles of bioethics: the primacy of a person, providing equal access to health care, the need for consent to medical intervention (and the protection of those who are unable to give it), confidentiality.

Transplantation of organs from a deceased person today is a more expedient and justified form of donation, because under such conditions the life and health of a living donor is preserved. It should be noted that the risk of postoperative complications for the donor is always quite high. In addition, organ transplantation from a deceased donor is less expensive, which is often of great importance to recipients. Postmortem transplantation allows for a greater number of transplants to recipients (one donor can give biomaterial to several patients at once) and to perform surgical interventions that are impossible from a living donor. Therefore, today, the increase in the number of surgical interventions for the transplantation of organs and cells should be carried out at the expense of the increase in the number of posthumous donors. The insufficient number of posthumous donors in Ukraine, in turn, necessitates the search for biomaterials in other countries [31]. International regulation of biomaterial exchange and transplantation operations is carried out on the basis of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [32]. In the mentioned Declaration, it was proposed to take measures to increase the efficiency of donation from the dead, as well as measures to combat transplant tourism, commercialization of transplantation, and organ trafficking; measures to ensure the protection and safety of donors' lives [13].

Secondly, the legislation of Ukraine provides for a limited list of persons who can become donors of organs or tissues. The existing list makes it impossible to donate between cousins, third cousins, godparents, as well as persons who are willing to become donors for people who are not relatives of the recipient. It is about the functioning of the so-called "family donation", to which only close relatives have the right to provide their biomaterial for transplantation. It is precisely such legal norms in Ukraine that force Ukrainians to use foreign medical services and, as a result, not to support the domestic medical sphere. It is a well-known fact that material from a living donor takes root better in a patient than the material from a cadaver donor. But the humaneness of replacing cadaver donation with an altruistic one remains an open question from the standpoint of human dignity, legal values, moral and ethical beliefs.

It should be noted that the procedure for granting the consent of the donor for the removal of biomaterials, the expression of will for the legal representative regarding the removal of implants from the body of the deceased in Ukraine is regulated by the Decree of the Cabinet of Ministers of Ukraine of December 27, 2018 No. 1211 "Some issues of implementation of the Law of Ukraine "On the Application of Transplantation of Anatomical Materials to Humans" [33].

Thirdly, it should also be noted the lack of sufficient control by law enforcement agencies over the transplantation institute. It is necessary to develop surveillance to identify and bring to justice persons who carry out "brokerage" or other illegal activities (especially through the Internet) in the field of extraction of anatomical materials for transplantation and manufacturing of bio-implants. A monitoring system for the detection of advertisements for the sale or purchase of human organs or tissues can be an effective mechanism for regulating the institute of transplantation.

As S. Grinchak points out, it is very difficult to bring to justice medical staff who perform actions in the field of transplantation that have signs of a crime. This indicates a high level of latency of such crimes and significantly complicates the assessment of their real state. And if we take into account that only a small part of criminal proceedings regarding crimes in the field of providing medical services come to the stage of trial, then we can conclude that the country does not have a sufficiently effective mechanism for combating such crimes [5].

Based on world experience, two options for the development of this institute can be identified:

– the first way is a person's lifetime consent to be a donor after death. Germany, Poland, France, and Spain have long used such a system, where a person is asked about the possible use of his or her organs after death while he or she is still alive.
– the second way is to introduce a system of "presumption of consent", when all residents of the country

are potential donors, but if they are not ready to be donors after death due to some moral-ethical, religious or other beliefs, they must notify the special institutions that put such persons into a special register.

Such systems have a number of advantages:

1) the lists of potential donors is formed.
2) a national register of persons who, during their lifetime, spoke against the transplantation of their organs in case of death is formed; this person's organs must not be removed under any circumstances.

For example, in Ukraine, in accordance with the Resolution of the Cabinet of Ministers of Ukraine dated December 23, 2020 No. 1366 "On approval of the Regulation on the Unified State Information System for Transplantation of Organs and Tissues" [34] there is the Unified State Information System for Organ and Tissue Transplantation operates, the task of which is to identify donor-recipient pairs, provide the participants of the national organ transplantation system with effective and expedient (in real time) information on potential donors of human anatomical materials, available human anatomical materials intended for transplantation and/or production of bio-implants, persons in need of medical assistance with the application of transplantation, persons in need of medical observation in connection with the transfer of transplantation, as well as other information necessary for the normal functioning of the transplantation system in Ukraine. Access to the registers of the Unified System is free.

The functioning of the system is provided at the expense of the state budget. The Unified State Information System of Organs and Tissues for Transplantation contains information on the voluntary consent of a person or his or her representative to the removal of anatomical materials or the manufacture of bio-implants, data on donors and recipients, information on medical institutions that have the right to real opportunities to perform transplant surgeries, data on transplants coordinators.

The first registry of cell donors appeared in the United States of America, the Americans were the first in the world to use the method of bone marrow transplantation. Initially, similar registries functioned separately in each state, but this made it difficult to find the necessary donor or material. In order to improve the search system, such separate registers were combined into the National Register of the United States of America. Over time, such information bases also appear in Europe, Asia, and Australia. However, the creation of domestic registries did not always provide an opportunity to find the right donor or anatomical material. This led to the creation of relevant international databases (for example, in Europe – European Marrow Donor Information System), and then the formation of a worldwide network – Bone Marrow Donors Worldwide.

European Marrow Donor Information System is an open network between different bone marrow donor registries for patients waiting for a transplant who do not have compatible family donors. The network consists of the same system in all countries. Email addresses are used as a method of communication. Bone Marrow Donors Worldwide is a voluntary joint association of bone marrow donor registries and umbilical cord blood banks to provide centralized and anonymous information on phenotypes, other data of unrelated bone marrow and umbilical cord blood donors; ensuring easy access to this information by doctors and recipients needing stem cell transplants. The system includes 51 bone marrow donor registries from 37 countries and 29 cord blood registries from 19 countries.

Separately, it is worth paying attention to the medical services that can be provided at the transplantation institute:

1) the first of them consists in the provision of medical assistance with the application of transplantation, that is, in fact, transplantation services (transplantation of anatomical material), which are provided in transplantation centers;
2) the second is the implementation of activities related to transplantation: medical services for the extraction of anatomical materials from living donors; removal of anatomical materials from a cadaver donor; storage and transportation of human anatomical materials intended for transplantation, storage and transportation of such materials for the manufacture of bio-implants.

The innovative provision of the Law of Ukraine "On the Application of Transplantation of Anatomical Materials to Humans" [25] allows health care institutions and scientific institutions of all forms of ownership to carry out transplantation activities today. Before the entry into force of the new law, such actions could only be carried out by state and communal health care institutions. The new law gives the right to all health care institutions that have a license to carry out economic activities in the field of medical practice (including the right to provide medical assistance with the application of transplantation and to carry out activities related to transplantation) to carry out such medical activities. This provision opens wide prospects directly for medical institutions and doctors working in private hospitals.

CONCLUSIONS

Organ donation is a legal relationship that has not yet been properly substantiated in legal and medical practice in Ukraine, and society's acceptance of the institute of transplantation as an innovative one is accompanied by many opposing views. The legal aspect of the study of the institute of transplantation is important for formulating the general patterns of its emergence, developing prospects for its functioning and strategic directions for further development, building a system for protecting the rights of all participants in this legal relationship.

When transplanting organs in humans, different aspects of the problem can be considered separately: medical or surgical, which includes the method and procedure of transplanting biological material; biological, which reveals compatibility or suppression, rejection of transplanted biomaterial by the human body; psychological, which answers the question of how to live a person with an implant in the future. But there is an equally important aspect – the legal one, which reveals the institution of transplantation from the procedure of conducting the procedure, providing medical services, protecting human dignity, and the rights of all participants in the legal relationship.

Transplantation is a modern challenge to the civilized world. Members of world society must realize the need for donation today, as a component of modern medicine, as a means of treating diseases incurable by other means, a person must understand that his or her organs after death can save someone's life. The discussion of this issue in society can contribute to the fact that the lists of potential donors will increase along with the number of consents given for the removal of donor material from a relative after death for transplantation, which in turn will give a sick person a chance to prolong his or her life.

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CASE STUDY

MARBLE DISEASE (CASE REPORT)

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ABSTRACT

We present clinical case of marble disease in 5 yo girl. The management of this child was made in Vinnitsia Regional Children's Hospital (Vinnitsia, Ukraine). CBC, X-ray of bones, bone marrow biopsy, genetical testing, MRI of the brain and CT of the skull were done during this period. Marble disease is a very rare disease with very serious consequences, the prevention of which requires timely diagnosis and treatment, namely the prevention of infectious complications and early allogenic transplantation of stem cells. As it is a genetically determined disease, it is not possible to prevent the development of osteopetrosis. Genetic screening and proper treatment will allow the patient to lead an almost normal life.

KEY WORDS: marble disease, children, clinical presentation

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INTRODUCTION

Marble disease (osteopetrosis, Albers-Schoenberg disease) is a very rare inherited disease characterized by high bone density due to mutations that affect the formation or function of osteoclasts. There are two main clinical forms, namely benign autosomal dominant (adult) and malignant autosomal recessive (infantile). The prevalence is 1 case per 100 thousand population and occurs due to osteoclast dysfunction, which causes the development of osteosclerosis [1, 2]. Autosomal recessive (intermediate) form is very rare. Diagnosed in childhood and has manifestations of malignant disease. The vast majority of young patients with malignant osteopetrosis die at an early age, but the development of medical science has made it possible to improve the quality of care for these patients, which has extended survival [3].

It should be noted that there is a secondary osteopetrosis, for example, associated with viral hepatitis C, which is characterized by a generalized increase in bone mass and a significant increase in the level of alkaline phosphatase in the serum. This form of the disease occurs in the elderly and is not described in pediatric patients. Despite significant progress in the development of genetics, a significant proportion of patients remain undiagnosed [4]. Histological examination may reveal the complete absence of osteoclasts in the bone biopsy or, conversely, an increase in the number of multinucleated osteoclasts on the background of spongy bone sclerosis with increased mineralization of the bone matrix [5].

The most common cause of children's malignant marble bones believed mutation gene TCIRG1, but the numerous studies revealed mutations of additional genes and is TNFSF11, TNFRSF11A, CLCN7, OSTM1, SNX10, PLEKHM1, which is also associated with autosomal – recessive form of marble disease [6]. In order to diagnose these disorders in malignant infantile osteopetrosis use sequestration of the whole exome, and mutations are detected in four genes: CLCN7, TCIRG1, SNX10 and TNFRSF11A [7].

But in about 20% of cases the molecular basis is unknown. It is described that the key regulator of bone remodeling is NF-kB signaling. Recent results have shown that inactivation of NF-κB enhances osteoblast differentiation in vitro and bone formation in vivo. The complex of this transcription includes five subunits, such as p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). One of them, namely Rela/p65, is very important within the NF-kB pathway for bone homeostasis and may be one of the genetic markers of Marble Disease [8, 9].

Usually, the first clinical sign of the disease are fractures that occur spontaneously or with minor trauma, followed by slow and incorrect consolidation of the fracture. In addition, detect hematological disorders, neurological disorders and delayed psychomotor development [10]. The literature describes the correlation between radiological data and mutations in genes. Thus, patients with TCIRG1 and RANK mutations often have pathological fractures, in addition to hepatosplenomegaly and hydrocephalus. Varus deformity of the femoral neck is more typical for patients with TCIRG1 mutation [11]. Children with

Table I. Indicators of peripheral blood in dynamics:

Date	HB (g/l)	RBC ($\times 10^{12}$/l)	WBC ($\times 10^{9}$/l)	Platelets (thousand/mm^3)
05/07/2018	64	2.29	6.89	312
02/26/2019	52	1.90	20.0	52
05/08/2019	42	1.20	12.4	19
07/17/2019	71	1.46	10.5	58
10/08/2019	63	1.46	13.0	71
01/20/2020	49	1.32	11.4	21
04/03/2020	39	1.12	16.9	19
08/09/2020	52	1.41	13.4	28
11/27/2020	55	1.56	14.1	34
01/23/2021	24	0.90	20.2	68
05/09/2021	37	1.12	22.7	34

malignant osteopetrosis also have impaired tooth development (delayed or failed eruption, improperly formed crowns and roots, enamel hypoplasia) and jaws, which often causes osteomyelitis, which is usually difficult to treat and can be fatal. Therefore, prevention and treatment of oral infection requires constant supervision of a dentist and maxillofacial surgeon [12]. Due to the disorder of resorption of osteoclasts, there is a violation of bone marrow formation, which leads to defective hematopoiesis and mortality during the first ten years of life. The only currently available treatment for some forms of autosomal recessive osteopetrosis is allogeneic hematopoietic stem cell transplantation (allogenic transplantation of the stem cells) from a familial or nonfamily donor. The best results are achieved in children in the first year of life, as the residual volume of the bone marrow cavity decreases over time. Magnetic resonance imaging (MRI) should be used to assess the bone marrow cavity, which also allows to assess skeletal reconstruction after transplantation and the consequences of changes in the hematopoietic system [13]. Despite the increase in the overall survival of patients after allogenic transplantation of the stem cells, the issue of treatment tactics remains unresolved. The problem is the search for a compatible donor and the presence of neurological symptoms, which significantly worsens the prognosis. Today, new treatments are being sought, preclinical studies are being conducted, such as gene therapy, systemic administration of deficient protein, intrauterine allogenic transplantation of the stem cells and correction of defects in genes [14].

CASE REPORT

A girl, 5 years old (born on February 24, 2016) is often hospitalized in the oncohematology department of Vinnytsia Regional Children Hospital due to severe anemia, which requires replacement blood transfusion with erythrocytes. First time she came to the clinic on May 7, 2018 with complaints on pale skin, inhibition, the appearance of multiple bruises on the skin, fever.

The child lives with his mother, who has a significant delay in mental development, there were no pathological changes during the physical examination.

Examination of the child revealed a delay in physical (subnanism) and mental development. The girl is calm, has no contact with others, has a small vocabulary. The pronounced pallor of the skin and mucous membranes attracted attention. Hemorrhagic syndrome with polychrome and polymorphic rash on the skin of the face, chest, extremities. Peripheral lymph nodes are not enlarged. At auscultation of lungs – vesicular respiration. RR 20 per min, SpO2 98%. Heart sounds are muffled, tachycardia – 140 beats per minute, and a systolic murmur was heard above the apex. The abdomen is enlarged, the liver is +2 cm, the spleen is +3 cm from the edge of the costal arch, peristalsis is active. Urination is free, urine is light in color. Stools are decorated.

The child was examined by related specialists:

- Medical genetic – Alberts-Schoenber disease (marble disease);
- Tuberculosis specialist (phtisiatrist): data for specific pathology in the lungs was not detected;
- ENT: Acute rhinitis;
- Ophtalmologist: fundus within normal limits;

X-ray examination of skeletal bones was performed and the presence of diffuse osteosclerosis in the bones of the skull and extremities, which is characteristic of marble disease (Figure 1-8).

The girl was repeatedly examined for bone marrow.

Cytological examination of the bone marrow (08.05.2018): Bone marrow punctate small cell, hematopoiesis of the normoblastic type. The calculation was performed on 200 classes. When counting at low magnification in a smear 2 megakaryocytes.

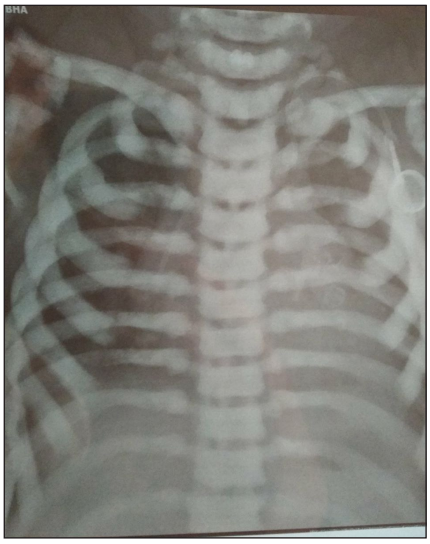

Fig. 1. Chest X-ray of the patient with Marble disease

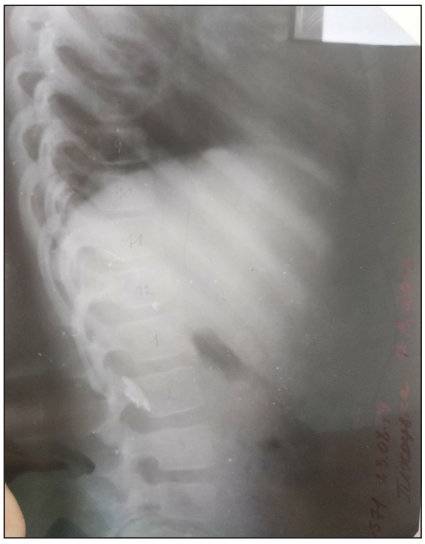

Fig. 2. Sided chest X-ray of the patient with Marble disease

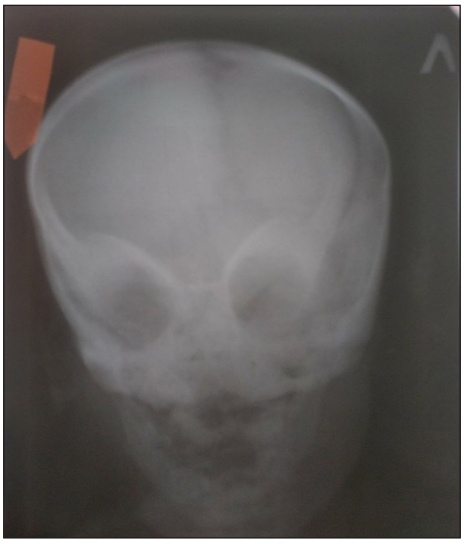

Fig. 3. Scull X-ray (frontal view)

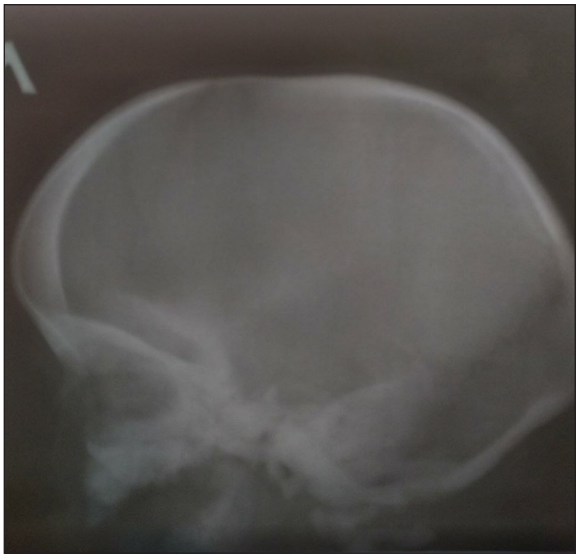

Fig. 4. Scull X-ray (sided view)

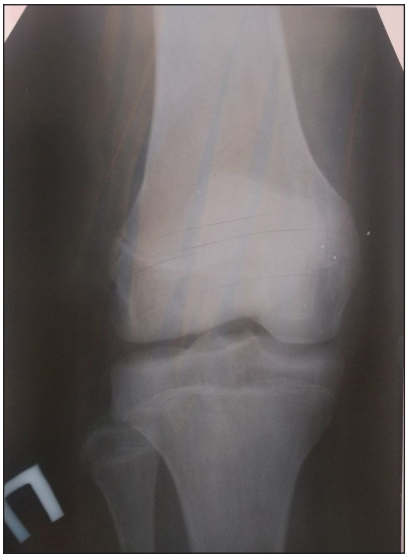

Fig. 5. X-ray of the right knee (frontal view)

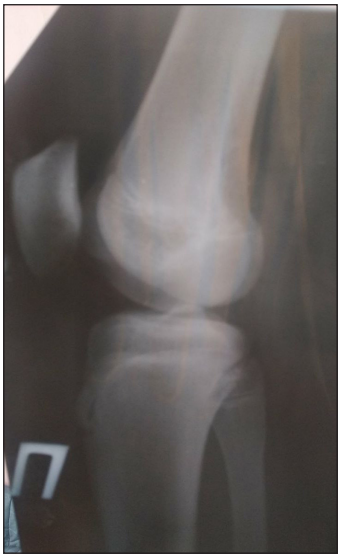

Fig. 6. X-ray of the right knee (sided view)

Cytological examination of the bone marrow (28.02.2021): Counting due to high cellularity, hematopoiesis of the normoblastic type. Granulocyte, erythroid sprouts – dyspoesis; megakaryocyte – suppression; blasts – 0.6%; the relative number of lymphocytes increased to 23.2%.

Peripheral blood indicators in dynamics are shown in Table 1.

On January 21, 2020, a genetic study was performed and a mutation in the CLCN7 gene was detected, which may indicate an autosomal recessive form of marble disease.

In January 2021, the mother reported that the child's eyesight had deteriorated and there was periodic twitching of the limbs. The girl was consulted by an ophthalmologist – atrophy of the optic nerve of both eyes was detected. MRI of the brain was performed, followed by examination by a pediatric neurologist: atrophy of the cerebral cortex.

In March 2021, there was a facial deformity (figures 9, 10). Osteomyelitis of the jaw was suspected.

On March 10, 2021, a CT scan of the skull and brain was performed. Conclusion: CT signs of hyperostosis with diffuse compaction of skull bones, which is most characteristic of marble disease, pathological fracture along the right maxillary suture with diastasis at its level and signs of osteomyelitis at its level, with edema and infiltration of the soft tissues of the right nose. -orbital area and right cheek with right exophthalmos; CT signs of dilatation with slowed blood flow through the venous sinuses of the brain, bilateral hemispheric cerebellar edema.

The child receives symptomatic treatment: erythrocyte transfusion, platelet apheresis, antibacterial therapy according to the indications. The documents were considered by the Multidisciplinary Commission

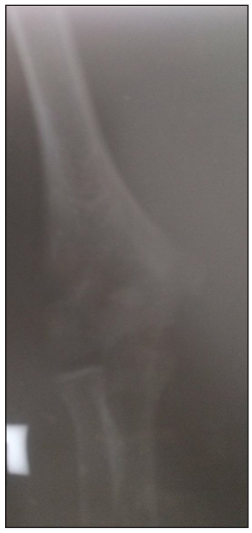

Fig. 7. X-ray of the right elbow (frontal view)

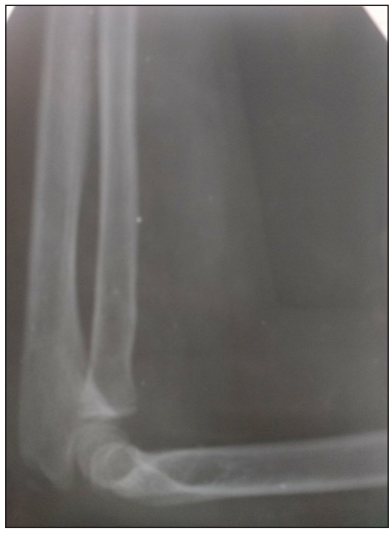

Fig. 8. X-ray of the right elbow (sided view)

of National Child Clinic "OKHMATDIT" in Kyiv, the indications to stem cells transplantation were not established.

DISCUSSION

Osteopetrosis belongs to the group of metabolic diseases of the skeletal system, which disrupts the reconstruction and growth of bone tissue, leading to generalized osteosclerosis and predisposition to pathological fractures. In addition, there is bone marrow failure due to ossification of the bone marrow canal, manifested by peripheral pancytopenia. This pathology was first described by the German radiologist Alberson-Schoenberg in 1904, who described the radiological picture of increased bone density due to osteoclast dysfunction [15]. Due to abnormal modeling and remodeling of the skeleton, mineral homeostasis is disturbed, which leads to deformation of the tubular bones and upper and lower jaws with impaired tooth growth. The growth of bone tissue in the bone marrow canal reduces bone marrow cellity and activates extramedullary hematopoesis, which is manifested by hepatosplenomegaly. Compression of the cranial nerve openings leads to blindness, deafness and paralysis [16].

There are three forms of osteopetrosis: benign autosomal dominant, malignant autosomal recessive and intermediate autosomal dominant. According to statistics, the prevalence is 1 in 100,000 – 500,000 cases [1]. The autosomal recessive form of osteopetrosis is known as childhood malignant osteopetrosis and occurs in 1 in 250,000 newborns. Most often, children with related pathologies are born with this pathology. Much less – in families, where parents found a defective gene. Autosomal recessive osteoporosis

Fig. 9. Photo of the child with marble disease

Fig. 10 . Photo of the child with marble disease

related to the presence of mutations in different genes are involved in functional capacity and osteoclast differentiation. Stigmas of dysembryogenesis, skull abnormalities and teething, hypocalcemia are observed in children. The only method of treatment of this form – THC, without which there is mortality up to 10 years of age [17].

The most common form of autosomal recessive osteopetrosis is caused by a mutation in the TCIRG1 gene. TCIRG1-mutated osteoclasts have significantly reduced resorptive capacity. In addition, this form is characterized by the development of rickets and osteomalacia, due to impaired absorption of calcium in the stomach [18]. The second frequency autosomal recessive form connected with mutations in the CLCN7 gene. In the presence of this mutation, a wide range of clinical manifestations is observed. Patients have a very severe course of the disease, with skeletal defect and bone marrow failure combined with primary neurodegeneration, reminiscent of lysosomal storage diseases, cerebral atrophy, hyperkinesia, axial hypotension and peripheral hypertension [19]. Much less is observed austosomal recessive marble bones, related mutations in OSTM1.

However, it is associated with primary severe neurodegeneration and a life expectancy of less than 2 years [20]. The complete absence of osteoclasts is the main feature of the form depleted of osteoclasts. It is caused by the absence of RANKL and is encoded by the TNFSF11 gene. Patients have impaired T cell synthesis and cytokine production. In addition, reduces the formation of B cells and the synthesis of immunoglobulins [21]. There is also a report of a heterozygous mutation in the CSF1R gene, which encodes the MCSF (macrophage colony-stimulating factor) receptor, which is found in related children and is manifested by osteopetrosis with brain malformations [22]. This disease should be differentiated from other metabolic diseases characterized by fragility of the skeletal system, such as rickets, incomplete osteogenesis, Ehlers-Danlos syndrome, Marfan syndrome, hypophosphatemia and skeletal fluorosis [23].

The literature also describes the acquired form of marble disease, which occurs when fluoride accumulates in bone tissue (fluorosis), which leads to a pronounced density and fragility of bones. In addition, there is a localized form of osteopetrosis, which occurs in patients with malignant neoplasms that metastasize to bone tissue [24]. There is a benign form of osteopetrosis that manifests in adolescence. Based on biochemical, clinical and radiological features, two types are described: type I and type II. Severe course of the disease is observed in type II and is characterized by severe osteosclerosis of the skull and spine, high risk of pathological fractures and a significant increase in the level of acid phosphatase in the serum.

Patients need symptomatic treatment as there is no specific therapy [25]. Treatment of marble disease requires a multidisciplinary approach, given the presence of neurological symptoms, bone defects, bone marrow failure, metabolic disorders and susceptibility to infectious complications. THC improves the 5-year survival of patients, but it must be performed before the onset of irreversible neurological disorders. Describe the use of 1b (IFNγ1b) gamma interferon before bone marrow transplantation, which improves the immune response, enhances bone resorption, increases the lumen of the bone marrow canal [26].

CONCLUSIONS

Marble disease is a very rare disease with very serious consequences, the prevention of which requires timely diagnosis and treatment, namely the prevention of infectious complications and early allogenic transplantation of stem cells. As it is a genetically determined disease, it is not possible to prevent the development of osteopetrosis. Genetic screening and proper treatment will allow the patient to lead an almost normal life.

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