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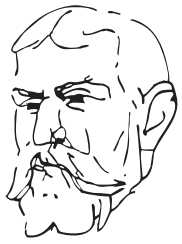

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ORIGINAL ARTICLE
PRACA ORYGINALNA

THERMOGRAPHIC DIFFERENTIAL DIAGNOSIS OF ACUTE TONSILLITIS AND EXACERBATION OF CHRONIC TONSILLITIS

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ABSTRACT

The aim: To elaborate the thermosemiotics of acute and chronic tonsillitis during exacerbation.

Materials and methods: 48 patients with acute tonsillitis and 19 patients with chronic tonsillitis were examined during disease exacerbation. Thermographic examination was carried out by the medical thermometer TI-120. Statistical processing of data was carried out with computer program "Microsoft Excel" and "Statistica for Windows" v. 6.0, StatSoft Inc. (USA).

Results: Symmetry, homogeneity and isotherm are normal thermographic features of the skin. Presence of hyperthermia in carotid triangle, on the palms were revealed in patients with acute tonsillitis. In chronic tonsillitis in the stage of exacerbation, a moderate overall «warming up» of the neck and projections of the submandibular lymph nodes was also observed. The average temperature indices of the knee joints were statistically significantly higher than the corresponding values in healthy people °C ($P<0.01$).

Conclusion: Only in patients with acute tonsillitis clear strong positive correlation between the temperature of half of the submandibular area and warming in the center of the palm – $r = 0.812 ... 0.859$ ($P<0.01$) and in the middle of the tenar on the corresponding side – $r = 0.790 ... 0.827$ ($P<0.01$) was observed. Only in the case of chronic tonsillitis, in the stage of exacerbation, the temperature difference over the knees and the lower part of the femur is always greater than 0.5 °C.

KEY WORDS: acute tonsillitis, chronic tonsillitis, thermography, differential diagnosis

Wiad Lek. 2020;73(3):417-422

INTRODUCTION

Acute tonsillitis is a rapid onset infectious inflammation of tonsils that is the third most common infection after influenza and other acute respiratory infections in etiological structure of infectious morbidity [1, 2].

Cases of Streptococcal infection are comprised of more than 50 % of a total number of patients with respiratory infections [3]. It is important that after acute tonsillitis relapsing inflammation of palatine tonsils may be formed. It is favorable for formation of tonsillar diseases (rheumatic fever, myocarditis, glomerulonephritis, pyelonephritis, etc.). These data point out tonsillitis as actual problem of theoretical and practical medicine.

In the last 10 years, the diagnosis of "tonsillitis" became widespread both in Ukraine and in the world.

According to World Health Organization (WHO) data among the world's population about 616 million cases of streptococcal tonsillitis are diagnosed annually [4]. Researches that are carried out in different countries indicate an increase of the cases of streptococcal infections in the human population and the appearance of severe cases of the disease due to the variability of the pathogen [5-8]. Streptococcal infections remain one of the relevant reasons of the population morbidity which allowed WHO to name them an actual medical, social, and economic public health issue [9,10]. The relapsing course of streptococcal tonsillitis and it's frequent complications despite of the proper etiotropic therapy allow to hypothesize about existence of unstudied mechanisms in this infectious disease.

The thermographic criteria of acute and chronic tonsillitis in the stage of exacerbation concerned the features of the thermorelief of the anterior half of the neck were described previously. Thus, according to the known method (in a known way), in patients with acute process, a spilled hyperthermia in the projection of the tonsils and submandibular lymph nodes is appeared, but temperature difference with adjacent regions (ΔT) usually exceeds 0.6 °C, and in chronic cases, limited in area hyperthermia in the upper part of the neck concerning to the type of "collar" with ΔT in most patients is 0.3-0.6 °C [11, 12].

However, this method is not sufficiently precise and specific, since the degree of hyperthermia and the area of intensive light directly depend on the level of inflammation in the tonsils, which alleviates the specified thermometric boundaries between acute and chronic tonsillitis.

THE AIM

To elaborate more clear (or clearer) and specific the thermosemiotics of acute and chronic tonsillitis in the stage of

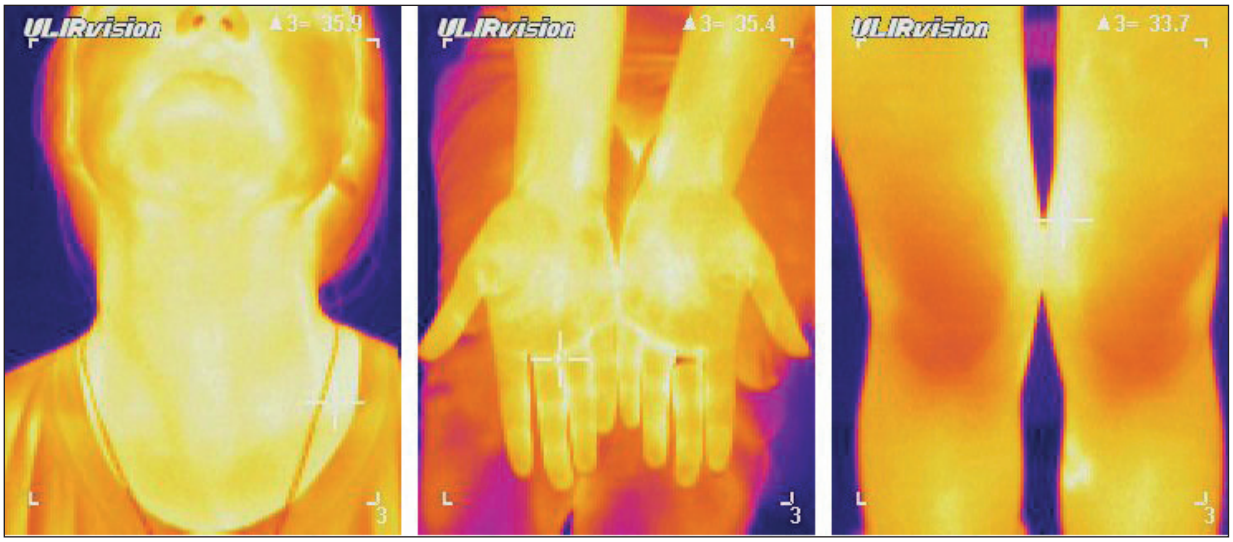

Fig. 1. Patient S.G, 28 years old, with acute tonsillitis. Isothermal temperature distribution of the of the skin of the subnundibullar region, palm, and knees

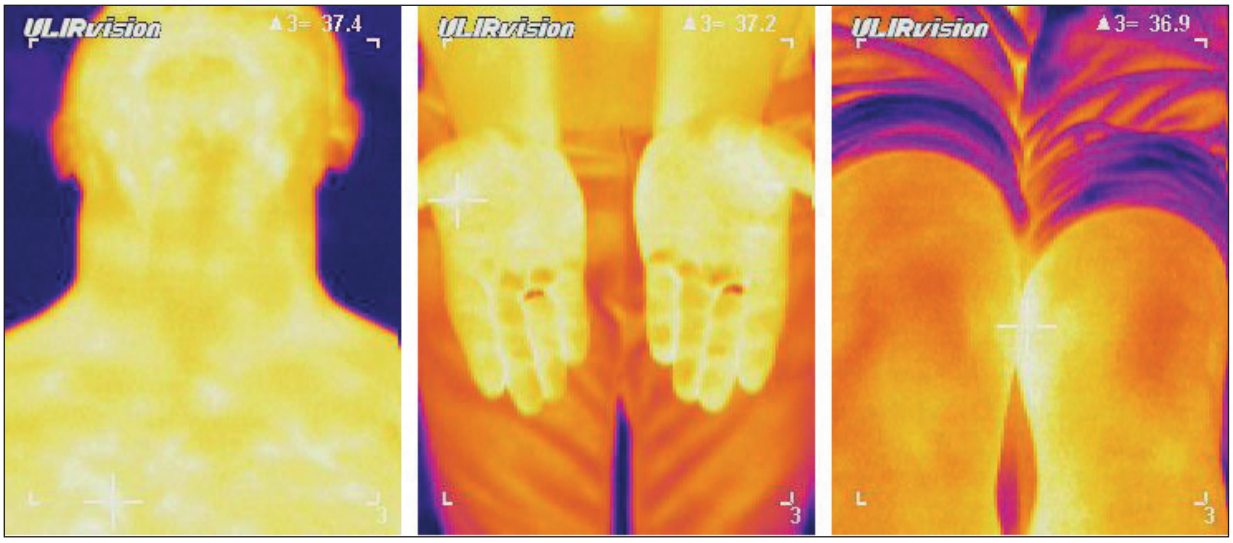

Fig. 2. Patient V.P. 34 years old with acute tonsillitis. Hyperthermal symmetrical temperature distribution of the of the skin of the subnundibullar region, palm, and knees

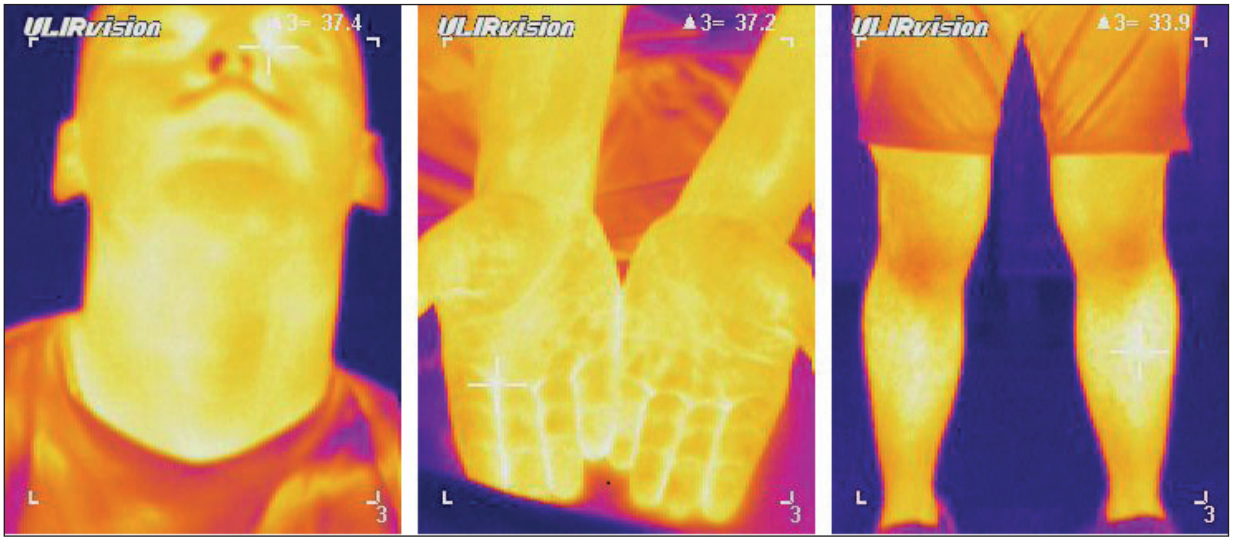

Fig. 3. Patient Ya.N. 24 years old with acute tonsillitis. Hyperthermal asymmetrical temperature distribution of the of the skin of the subnundibullar region, palm, and knees

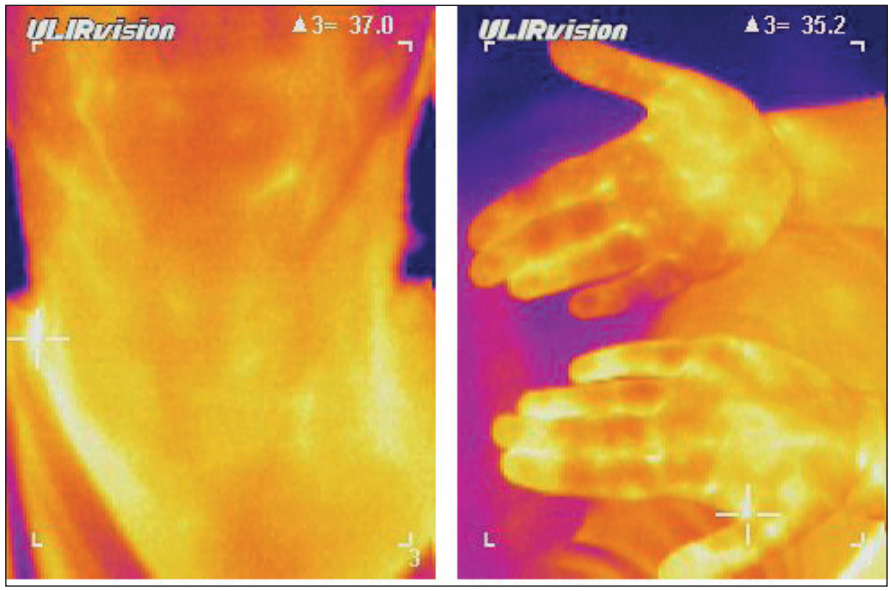

Fig. 4. Thermogram of the anterior part of the neck and palms of patient M.V., 39 years old with chronic tonsillitis in the stage of exacerbation, decompensated form (hyperthermia in the projection of a carotid – ΔT = 0.6 °C and in the middle of palms – ΔT = 1.1 °C)

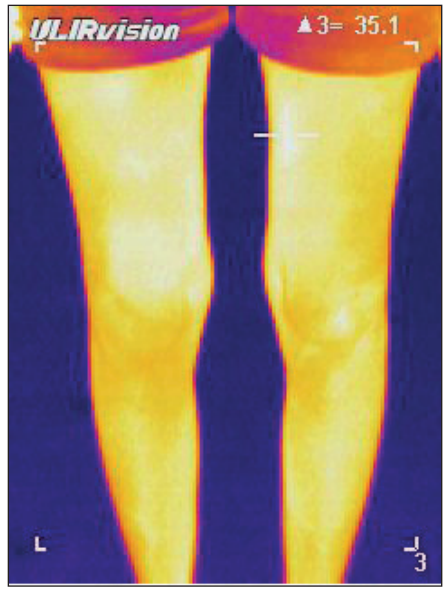

Fig. 5. Termogram of the leg of the same patient (knee hyperthermia – ΔT = 1.9 °C)

exacerbation by determination of the characteristics of infrared radiation in various anatomical sites of such patients.

MATERIALS AND METHODS

To determine the distinct characteristics of infrared radiation in the submandibular region and on the palms, 48 patients (26 men and 22 women) with acute and 19 (8 men and 11 women) with chronic tonsillitis in the stage of exacerbation were examined. Their age was between 23 and 58 years with average year (36.8±13.5). Control group consist of 32 healthy volunteers of the same age with normal body temperature and they had no history of tonsillar pathology. The diagnosis of acute bacterial tonsillitis was made using clinical, laboratory, and instrumental examinations.The survey was conducted at the permission of the Bioethics Commission of I.Ya. Horbachevsky Ternopil State Medical University (Minutes No. 125/18 dated September 26, 2018).

Hyperemia, bloster-like thickening of the edges of the palatine arches, cicatrix comissures between the tonsils and palatine arches, caseous and purulent corks in the tonsillar lacunas, regional lymphadenitis, tonsilogenic intoxication and prolonged subfebrile conditions, as well as anamnestic data about manifestations of decompensation in the form of frequent recurrent acute tonsillitis for a long time. Thus, the signs of chronic tonsillitis in the stage of exacerbation were and reticulum cicatricial adhesions between loose tonsils and palatine arches, cystic-purulent cork, regional lymphadenitis, tonsilogenic intoxication were the main clinical signs of chronic tonsillitis in the stage of exacerbation. To determine the reliability of streptococcal etiology of tonsillitis, the Centor scale of clinical symptoms evaluation was used [13]. Presence of paratonsillitis, parontosillar abscess and other complications, as well as concomitant inflammatory diseases, were the criterion for exclusion of person from the study.

Thermographic inspection was performed using medical thermal imager TI-120. Analysis of thermograms was carried out with the program package "IRSee Software". Thermal images were assessed visually by comparison of the color palette with nearby regions. If there was a thermal asymmetry, thermograms were described by the following algorithm: presence of thermal asymmetry; localization of the zone with increased and reduced intensity of infrared radiation; temperature values and their difference from a symmetrical zone. The thermographic survey of the submandibular region and neck was performed on the patients in sitting position. Apparatus was focused at a distance of about 40-50 cm from the face in facial projection when the head was tilted back. We paid special attention at a carotid triangle that corresponds to the position of the carotid artery and submandibular lymph nodes, the projection of the larynx and head-nod muscles. Keeping the same distance and projection, thermograph of palms was made. At the same time a temperature of the thenar, finger-tips, hypothenar and the middle of the palm was evaluated.

Statistical processing of data was carried out with computer program "Microsoft Excel" and "Statistica for Windows" v. 6.0, StatSoft Inc. (USA).

RESULTS

It is known in healthy persons, the temperature distribution of the right and left submandibular regions and both of the palms and knees are symmetrical according to average indices [12]. Mild hypothermia in the projection of the larynx and head-nod muscles, hyperthermal cords along the neck's magistral vessels were the characteristic thermographic features of submandibular regions. The thermal radiation above this zone in absolute figures fluctuated between minimum and maximum values of – 34.2-36.8 °C, with average level (35.1±0.7) °C. In general, following are the features of normal palm and knee teplovision visualization:

Table I. Distribution of average temperature indices in different anatomical regions in healthy persons and patients with acute tonsillitis in the stage of exacerbation

Anatomical region	Average temperature (M±m) °C		
	healthy persons (n=32)	acute tonsillitis (n=48)	chronic tonsillitis (n=19)
Carotid triangle	35.1±0.7	37.5±0.8*	37.1±0.6*
Thenar	34.0±0.6	36.9±0.6*	35.3±0.6
Middle of the palm	34.2±0.6	37.0±0.7*	35.7±0.4*
Knee joints	32.9±0.5	33.2±0.4	35.5±0.7*

Note * – a reliable difference compared to a group of healthy individuals (P<0.05-0.001).

Table II. Results of thermal imaging inspection of carotid triangle, palms, and legs in patients with chronic and acute tonsillitis in stage of exacerbation

Presence of hyperthermia (ΔT °C)	Tosillitis	Locus							
		carotid triangle		thenar тенар		midle of the palm		knee joints	
		abs. number	M%±m%	abs. number	M%±m%	abs. number	M%±m%	abs. number	M%±m%
0-0.5 (Norm control)	Acute (n=48)	3	6.3±3.5	0	0.0±0.0	0	0.0±0.0	48	100.0±0.0
	Chronic in stage of exacerbation (n=19)	2	10.5±7.0	3	15.8±8.4	2	10.5±7.0	0	0.0±0.0*
0.6-1.1	Acute (n=48)	16	33.3±6.8	9	18.8±5.6	10	20.8±5.9	0	0.0±0.0
	Chronic in stage of exacerbation (n=19)	11	57.9±11.3	7	36.8±11.1	6	31.6±10.7	3	15.8±8.4
1.2-1.6	Acute (n=48)	19	39.6±7.1	22	45.8±7.2	19	39.6±7.1	0	0.0±0.0
	Chronic in stage of exacerbation (n=19)	4	21.0±9.3	5	26.3±10.1	8	42.1±11.3	5	26.3±10.1*
>1.6	Acute (n=48)	10	20.8±5.9	17	35.4±6.9	19	39.6±7.1	0	0.0±0.0
	Chronic in stage of exacerbation (n=19)	2	10.5±7.0	4	21.1±9.4	3	15.8±8.4*	11	57.9±11.3*

Note. * – a reliable difference at compared with patients with acute tonsillitis in the border of proper range ΔT (P<0.05-0.001).

symmetry and homogenicity, isotherm with moderate relative hyperthermia of the middle of the palm, fingers of the hand and foot are traced to the fingertips The range of absolute values of palm temperatures was 32.3-34.5 °C, average figures – (34.2±0.7) °C. Although in 53 % of healthy individuals, thermal asymmetry can be registered at 0.2-0.6 °C with predominance in both the left and right side. Significant differences between male and female were not revealed [14-17]. We found previously that the temperature indices in healthy 20-40 years of age persons were significantly higher by 0.4-0.5 °C than in eldery persons (>40 years) [12].

Catarrhal inflammation was diagnosed in 3 of examined patients (6.3 %) with acute tonsillitis, follicular – in 11 (22.9 %), and 23 persons (47.9 %) had lacunar inflammation .

According to the character of thermal radiation in patients with acute tonsillitis we distinguished two states – isothermal (19 individuals) and hyperthermic (39 individuals).

In isothermal state temperature distribution in the tissues of the submandibular region and palms, and knees did not differ from the thermogram in control.

Taking into consideration the existence of physiological temperature asymmetry ±0.3 °C between the right and left half of the submandibular region, the palms, and knees such cases of temperature distribution were considered as symmetrical. Figure 1 illustrates these type of heat radiation.

The obtained combination of temperature indices on the histogram and the line of projection of both tonsils proves the absence of differences of thermal radiation in symmetrical regions.

Hyperthermic radiation, is a result of the inflammatory process in the tonsils, which is projected on the submandibular region.

It is important that out of 39 patients with this type of thermal radiation only in 13 persons local hyperthermia was approximately symmetrical (the temperature difference in the pair of parts – ΔT – did not exceed 0.2 °C) (Fig. 2), while in the rest – the temperature asymmetry reached even 1.2 °C (Fig. 3).

It is characteristic that despite of patients' age, in all these cases, the noted difference of temperatures in the carotid triangle, on the palms, primarily in their center and in the middle of the elevation of the thumb-thenar was observed. It is important that the temperature of these areas statistically significantly exceeds the proper value in healthy persons (P<0.05-0.001). At the same time the temperature of the knee joints did not change (Table 1). It was clear strong positive correlation between the temperature of the half of the submandibular region (the projection of the one of the palatine tonsils and the warming in the middle of the palm (r = 0.812 ... 0.859 at P<0.01) and in the middle of the thenar on the corresponding side – r = 0.790 ... 0.827 at P<0.01.

In 7 out of 19 patients with chronic tonsillitis in the stage of exacerbation there were compensated, and in the remaining 12 persons –decompensated forms of inflammation.

In exacerbation stage of chronic tonsillitis in thermotopogram of anterior part of the neck showed a moderate warming of the neck, a symptom of a "collar", where, on the background of insignificant hyperthermia brighter focal luminosities in the projection of lymph nodes, mainly submandibular ones were revealed (ΔT = 0.6 °C, table I. Fig. 4). At the same time, the average temperature indices of the knee joints were statistically significantly higher than the proper values in healthy persons – (35.5±0.7) vs. (32.9±0.5) °C (P<0.01). At the same time, other clinical signs of gonitis persecution were excluded.

Table II shows the fact presence fact of hyperthermia in the carotid triangle, on the patients' thenar and in the middle of the palms in absolutive vast of patients with acute tonsillitis, and ΔT usually exceeds 0.5 °C, and in every fifth to third – even 1.6 °C. At the same time, no one of these patients was registered warming the knee joints, since this area was practically isothermal with the lower part of the femur (ΔT does not exceed 0.3 °C). Similarly, on the thermograms of the anterior half of the neck of patients with chronic tonsillitis in the stage of exacerbation hyperthermia of the submandibular area and palms was dominant (ΔT was predominantly in the range of 0.6-1.6 °C). At the same time, the most significant (>1.6 °C) exceeding of the temperature difference in the middle of the palms in comparing with adjacent areas was found only in (15.8±8.4) % of such patients, which was significantly lower than in persons with acute tonsillitis (39.6±7.1) % (P<0.05).

In this case, only in the patients with of chronic tonsillitis in the stage of exacerbation, the in comparison with the lower part of the femur (ΔT is always greater than 0.5 °C), thermographic inspection of the knees always showed their hyperthermia and in (57.9±11.3) % of these patients ΔT exceeded 1, 6 °C (Table II, Fig. 5)

Since one of the main pathogenetic links of chronic tonsillitis in the stage of exacerbation is the presence of tonsilogenic intoxication, it can lead to infectious and allergic inflammation of large joints [18].

Let be assume that the degree of hyperthermia of the knee joints reflects the activity of chronic tonsillitis. At the same time, when patient has acute inflammation of palatine tonsils, large joints are not affected.

Conclusion about the degree of compensation of chronic tonsillitis tonsils can be made according to the level of temperature difference over the knees and the lower part of the femur. Thus, in 10 out of 12 patients with decompensated form of tonsillitis, indicated ΔT (more than 1.6 °C), and in all 7 patients with compensated form – moderate (1.2-1.6 °C). It is important that even without clinical manifestations of joints inflammation, the thermovisual inspection makes it possible to establish initial manifestations of tonsilogenic intoxication in the form of insignificant hyperthermia (ΔT = 0.6-1.1 °C).

DISCUSSION

As well in response to penetration of the infectious agent, the inflammatory process develops in the pharyngeal tonsils, which is manifested by local circulatory disturbances, increase of vascular wall permeability, local immunosuppression and, as a consequence, activation of endogenous microflora with ~~e~~ a gradual development of acute inflammation in the tissue of the palatine tonsils [19]. We assume that the marked relationship between the temperature of the submandibular palms, and knees is associated with reflex autonomic changes that lead not only to the dysregulation of the vascular tonus, but also are display remotely from the locus morbi in the Ged zones. As it is known, in the pathogenesis of their formation takes part anatomical and functional (metameric) connection between the skin and inner organs through the segmental apparatus of the spinal cord. In this process, a certain role plays a functional state of the central nervous system.

From the modern point of view Ged zonse may be interpreted as areas with altered sensitivity of the skin and other tissues (muscles, bones) of a certain metamer, a complex vasomotor and motor-trophic reflex, which is believed to be a skin metameric projection of affected internal organ. In these zones tenderness to palpation, changes of trophicity, skin electric conductivity, perspiration, skin temperature and disorder of skin surface sensitivity as hyper- or hypoalgesia were revealed [20].

This assumption is supported by the study of H. Asada et al. (2003), who on the basis of a thermographic examination of 131 patients with chronic tonsillitis in the remission phase found that in 2-4 hours after mechanical massage of the tonsils, the temperature of the palms in some patients increased by ≥1 °C. It is interesting that in this group of patients more favorable outcome after tonsillectomy was observed. Therefore researchers suggest to use the thermographic technique and above mentioned provocative palmar test as a marker for prognosis of effectis of tonsillectomy [15]. The same authors point out that in the area between the shoulder girdle and submandibular region a line of the hyperthermia has distinct contours and resembles "the collar", which is a characteristic feature of the toxicoallergic form of chronic tonsillitis.

Thus, the proposed method provides an increase of the informativeness of the differential diagnostic study in patients with acute and chronic tonsillitis in the stage of exacerbation, as well as the possibility of establishing the form of chronic tonsillitis. The peculiarity of thermosemiotics of chronic tonsillitis in the stage of exacerbation established by us is protected by patent [21].

CONCLUSIONS

1. In patients with acute and chronic tonsillitis the isothermal and hyperthermal (symmetrical and asymmetric) thermal radiation of submandibular areas and palms may be distinguished. Only in patients with acute tonsillitis can be traced a clear strong positive correlation between the temperature of half of the submandibular area (the projection of one of the palatine tonsils) and warming up in the center of the palm – r = 0.812 ... 0.859 (P<0.01) and in the middle of the tenar on the corresponding side – r = 0.790 ... 0.827 (P<0.01) can be observed.
2. In patients with acute tonsillitis, the regions of knee joints are practically isothermal with the lower part of the femur (ΔT does not exceed 0.3 °C).

3. Only in the case of chronic tonsillitis, in the stage of exacerbation, the temperature difference over the knees and the lower part of the femur is always greater than 0.5 °C, and in (57.9±11.3) % of these patients ΔT exceeds 1.6 °C.
4. The degree of compensation for chronic inflammation of the tonsils can be estimated by the level of temperature difference over the knees and the lower part of the femurs: ΔT in the range 1.2-1.6 °C indicates the compensated form, and ΔT which is greater than 1.6 °C is usually a sign of the decompensated form of chronic tonsillitis.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

RESEARCH OF BIOLOGICAL PROPERTIES OF ENTEROVIRUS STRAINS ASSOCIATED WITH ISCHEMIC STROKE

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ABSTRACT

Introduction: The research of biological properties of enteroviruses associated with ischemic stroke (IS) allows us to identify their intratypic differences.

The aim: to identify genetic markers of strains of enteroviruses associated with IS.

Materials and methods: 11 strains of enteroviruses isolated from the serum of patients with IS were identified in the virus neutralization test. Genetic markers of isolated strains (A_{bent}, marker S, marker rct_{40}) were determined.

Results: Eleven strains of enteroviruses were isolated from the serum of patients with IS. Eight viruses: Coxsackie B viruses (serotypes 2, 3, 4) and ECHO viruses (serotypes 6, 9, 27 (two strains), 29) were identified in these strains. Other three strains of enteroviruses were unidentified.

Different combinations of genetic markers were found. Seven strains of enteroviruses (Coxsackie B2, B3, ECHO 6, ECHO 9, ECHO 27 (two strains) and one unidentified virus) had virulence markers: A_{bent}–, rct_{40}+ and S–. Three strains (Coxsackie B4, ECHO 29, one unidentified virus) had markers: A_{bent}–, rct_{40}+, S+. Another one unidentified virus had markers: A_{bent}+, rct_{40}+, S –.

Conclusions: All 11 isolates of enteroviruses associated with IS had rct_{40}+ marker, 10 of the 11 isolates had marker A_{bent}– and 8 of 11 isolates had marker S–. The research of genetic markers allows to perform typic and intratypic differentiation of strains of enteroviruses associated with the IS.

KEYWORDS: ischemic stroke, enterovirus, biologic properties of enteroviruses

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INTRODUCTION

Scientific interest in enteroviruses is dictated by an annual increase in the number of isolated types of enteroviruses, their poliorganic tropism as well as an expansion of the spectrum of diseases caused by them. Scientists have proved the pathogenetic role of enteroviruses in pathologies such as myocardial infarction, myocarditis, pericarditis, dilated cardiomyopathy, atherosclerosis, glomerulonephritis, appendicitis, hepatitis, pancreatitis, juvenile diarrhea [1, 2, 3].

As of today the role of enterovirus infections in the development of vascular pathology of the brain is urgent since these diseases compose from 30% to 50% of diseases of the cardiovascular system, among which the ischemic stroke (IS) plays a leading role [4]. Important risk factors for the development of the IS are the infectious and inflammatory processes that initiate the development of atherosclerosis [5]. The role of representatives of herpes simplex viruses of 1 and 2 types, varicella virus, cytomegalovirus, Epstein-Barr virus, adenoviruses, influenza viruseses, enteroviruses, Chlamidia pneumonie and their associations in the development of the IS has been proven. At the same time, the role of enteroviruses as a trigger factor in the development of the IS, their epidemiologically relevant serotypes and biological properties remains urgent and needs a further in-depth research [6, 7, 8, 9].

According to researchers findings, there are differences between pathogenic and apatogenic strains of enteroviruses [10]. Genetic markers of enterovirus virulence allow to identify their intratypic differences. After consideration of various genetic markers for determination of the enterivirus pathogenicity, we have chosen the following frequently used markers: the bentonite marker reflecting the affinity degree to bentonite (A_{bent}+ or A_{bent}–), the magnitude of enterovirus plaques under the bentonite nutrient coating (marker S) and a decrease in the ability of attenuated strains to rct_{40} (at 40 °C). Integrated use of genetic markers to determine the phenotypic properties and virulence of isolated strains of viruses is more rational method than the use of any marker alone [11, 12]. Such a phenotypic characteristic is required for the grouping of viruses associated with the IS.

THE AIM

The purpose of our research was to identify the genetic markers of strains of enteroviruses isolated from the sera of patients with the IS.

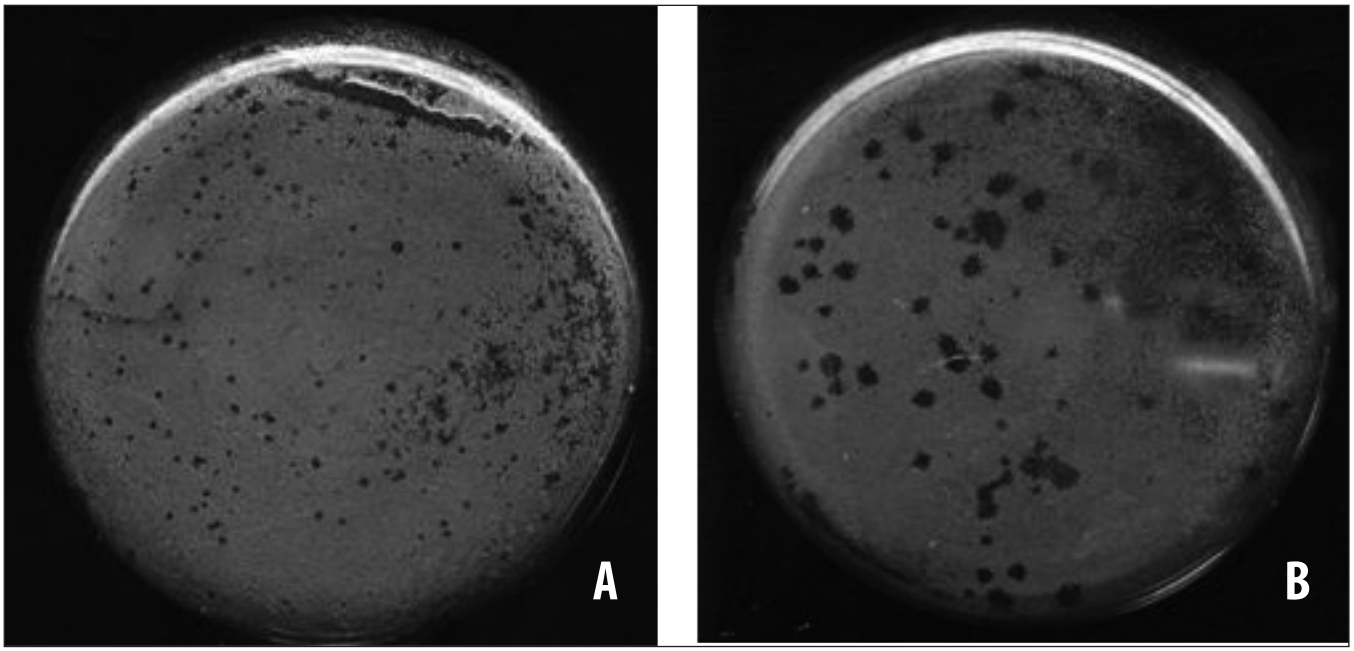

Figure 1. The reaction of plaque formation under bentonite nutrient coating in HEp-2 cell culture:
a) small plaques caused by the Coxsackie B3 virus;
b) large plaques caused by the Coxsackie B4 virus.

MATERIAL AND METHODS

The materials for the reserch were 11 isolates of enteroviruses isolated from the sera of patients with various forms of the IS, who were hospitalized in the neurological department and the department of cerebrovascular pathology of the Alexander Clinical Hospital in Kyiv in 2009-2016. Virus isolation was performed using the WHO recommended cell cultures: RD, HeLa and HEp-2. The identification of isolated viruses was performed using diagnostic sera for poliomyelitis of I-III types, Coxsackie B viruses of 1-6 types and ECHO of 1-34 types in the virus neutralization reaction. The genetic markers of isolated strains of enteroviruses were determined, namely: the bentonite marker A_{bent} which determines the affinity degree to bentonite; marker S determined by the size of viral plaques under the bentonite coating; marker rct_{40} which plays a role of an indicator of the viruses ability to be reproduced at elevated temperature (40 °C) according to the common method [10, 13].

RESULTS

11 cytopathic viral agents were isolated from the sera of patients with the IS in cell cultures by the classic virologocal method. The enterovirus genome was detected in polymerase chain reaction (PCR) [9].

The isolated virus strains were identified in the virus neutralization reaction as Coxsackie B viruses (serotypes 2, 3, 4) and ECHO viruses (serotypes 6, 9, 27 (two strains), 29). Other three strains were unidentified but detected as enteroviruses by PCR.

The selected virus strains had the following genetic markers: 10 out of 11 viruses had the A_{bent}– bentonite marker (90.9% of the strains) and only one of the unidentified viruses had the A_{bent}+ marker (9.1%). The greater virulence of the A_{bent}– of enteroviruses than A_{bent}+ variant was reconfirmed by the data obtained. A positive marker rct_{40}+ was detected in all strains of viruses (100%) isolated from the sera of patients with the IS. Virus-derived plaques were divided into two groups depending upon size: small plaques up to 1.5 mm in diameter (marker S–, 72.7%) and large plaques from to 1.5 mm in diameter (marker S+, 27.3%) (Figure.1)

When studying the genetic marker S under the bentonite coating, we isolated strains of ECHO 29, Coxsackie B4 and one unidentified virus strain. The mentioned three viruses induced the appearance of large plaques with festoon edges (marker S+). Majority of virus strains, namely: Coxsackie B2, Coxsackie B3, ECHO 6, ECHO 9, ECHO 27 (both strains) viruses, unidentified viruses (two strains) induced the appearance of small plaques with more even edges. That is to say, these viruses had the genetic marker S–. Hence, the strains of enteroviruses we have isolated induce the appearance of small plaques under the bentonite coating. According to V.P. Shyrobokov and V.N. Girin such appearance of small plaques is associated with the virulence of these strains and gives grounds to suggest about their role in the development of the IS [14].

It was proved by other researchers that marker rct_{40} has the highest correlation with enterovirus virulence while the bentonite marker A_{bent} and the marker S have a slightly less correlation [15]. According to our data, 7 strains of enteroviruses (63.6%) isolated from the sera of patients with the IS, namely: Coxsackie B2, B3, ECHO 6, ECHO 9, ECHO 27 (both isolates) and one unidentified virus, had all three positive virulence markers: A_{bent}–, rct_{40}+ , S– (induced

Table I. Distribution of genetic markers of virulence of enteroviruses isolated from the sera of patients with the IS

Viruses	Number of strains	Number of strains with genetic markers of virulence		
		A_{bent}–	rct_{40}+	S–
Coxsackie B	3	3	3	2
ECHO	5	5	5	4
Unidentified virus strains	3	2	3	2
Total	11	10	11	8

the appearance of small plaques in cell culture under a bentonite coating). Other strains of isolated enteroviruses were characterized by different combinations of virulence markers. In particular, Coxsackie B4, ECHO 29 and one unidentified strain had A_{bent}–, rct_{40}+, S+ markers; another one unidentified strain had A_{bent}+, rct_{40}+, S– markers (Table I).

Based on the results obtained, we can conclude about the phenotypic characteristics of the strains of selected enteroviruses by the genetic markers A_{bent}–, rct_{40}+, and S–. Therefore, the following characteristics are distinctive for strains of enteroviruses associated with the IS: the ability to reproduce at 40 °C (positive marker rct_{40}+), low affinity to bentonite (marker A_{bent}–) and the formation of small plaques under the bentonite coating (marker S–).

DISCUSSION

Bentonite marker (A_{bent}), first discovered and described by V.P. Shyrobokov, characterizes the degree of affinity of viral particles to bentonite. Particularly, it has been shown that A_{bent}– variant is the most virulent in the population of I type polioviruses and Coxsackie viruses while A_{bent}+ is more virulent in the polioviruses of II and III types [14]. Further studies revealed a dependence of bentonite affinity upon the type of viruses: polioviruses of all three types (vaccine strains), Coxsackie A7, A8, A18, Coxsackie B3, B4, B6 viruses have high affinity to bentonite. Coxsackie A10, Coxsackie B1 and B2 viruses have low affinity to bentonite [14]. A_{bent}+ and A_{bent}– dissociants differ in virulence, antigenicity and immunogenicity, organotropism and environmental stability [11, 16]. Highly virulent and immunogenic strains of enteroviruses have a low degree of affinity to bentonite at low alkaline pH and thus they have A_{bent}–. Our data is completely consistent with this finding. The bentonite test for intratypic differentiation of I type polioviruses also was recommended by L.V. Kopanitsa (2003). 84.9% of field isolates of polioviruses of I type which are of vaccine origin have a genetic marker A_{bent}+; and strains of polioviruses of I type with wild characteristics are represented by the variant A_{bent}– (100%) [16].

Therefore, the researchers consider the bentonite marker as an integral indicator that testifies a number of biological properties of isolated enterovirus strains and is used in the study of both clinical isolates and isolated enteroviruses from environmental objects (e.g. waste water) [12, 13].

The marker S was first described as a property of attenuated strains of polioviruses to form small plaques under agar coating. Method for detecting enterovirus plaques under a bentonite nutrient coating offered by V.P. Shyrobokov has appeared to be the most sensitive, faster, easier and more accessible in use than one under agar coating [14].

Thus, when studying the genetic marker S under the bentonite coating, we detected S+ marker in the strains of ECHO 29, Coxsackie B4 and one unidentified virus, whereas we detected the genetic marker S– in the other strains, namely: Coxsackie B2, B3, ECHO 6, ECHO 9 strains, ECHO 27 (both strains), unidentified viruses (two strains).

According to V.P. Shyrobokov, enteroviruses under bentonite coating can induce plaques that are heterogeneous in size. The differences in size of plaques induced by wild strains of polioviruses of II and III types and of plaques of their attenuated variants was proven [14]. Therefore, it can be stated that the marker S can also be used for intratypic differentiation of isolated enteroviruses.

Our data on the low ability of sorbtion to bentonite (A_{bent}–) in 10 of the 11 viruses (90.9%) isolated from the sera of patients with the IS are consistent with those of L.M. Grytsenko (2011), according to which a majority of strains of ECHO viruses isolated from patients (63.2%) had a low ability to absorb on bentonite (A_{bent}–) [17]. O.I. Yevtushenko proved that Coxsackie B viruses are mainly associated with the A_{bent}– genetic marker, which is able to overcome the placental barrier in pregnant mice and cause fetal and neonatal mouse deaths (91.2%), while variant A_{bent}+ causes the death of mice in 30.3% [18]. According to V.A. Ponyatovsky (2015), vaccine strains of polioviruses isolated from waste water had all negative virulence markers, the positive marker of S+ was most frequently detected in Coxsackie B; marker rct40+ was most frequently detected in ECHO; marker A_{bent}– was most frequently detected in the untyped viruses [12].

At the same time, according to our data, all 11 viruses isolated from the sera of patients with the IS had the marker rct_{40}+. This is consistent with the data of V.P. Shyrobokov who proved that more virulent Coxsackie B1 and poliovirus of II type MEF1 are more actively reproduced at higher temperatures in comparison with the low virulent Coxsackie B6 viruses and vaccine strain of poliovirus of II Sebin type [14]. L.M. Grytsenko (2011) found that ECHO virus strains isolated from healthy individuals (72.2%) were characterized by the rct_{40}– marker, and only 16.7% of the viral strains were characterized by the

rct_{40}+ marker. The majority of viral strains isolated from the patients had predominantly rct_{40}+ marker (73.7%), 10.5% of the strains had the rct_{40}– variant, and the remaining (15.8%) strains had the intermediate variant [17]. According to V.M. Girin, a high correlation between bentonite marker A_{bent} and rct_{40} marker was detected in 100% of strains isolated from waste water polioviruses as well as in 60.5% of ECHO viruses and 55.0% in Coxsackie viruses [16].

According to our data, the rct_{40}+ marker (all 11 isolates) was most frequently detected in the enterovirus isolates tested; the A_{bent}– bentonite marker (10 out of 11 isolates) and the S– marker (8 out of 11 isolates) were frequently detected.

Therefore, based on analysis of literature and the study of the genetic markers of enteroviruses isolated in sera of patients with the IS, we can confirm not only the virulence of isolated viruses, but also the presence of common phenotypic characteristics of viruses involved in IS. This allows us to recommend the determination of genetic markers for the intratypic differentiation of enteroviruses isolated from the sera of patients with the IS.

CONCLUSIONS

It was found that 11 isolates of enteroviruses were identified as Coxsackie B viruses (serotypes 2, 3, 4), ECHO viruses (serotypes 6, 9, 27 (two strains), 29) and three unidentified strains. They have certain genetic characteristics: the marker rct_{40} + was detected in all 11 isolates; 10 out of 11 isolates had the A_{bent}– bentonite marker; 8 of 11 isolates had the marker S– had. These common features can be considered as secondary characteristics for study of the enteroviruses role in development of the IS. The use of the virological diagnostic method with study of genetic markers allows to perform typic and intratypic differentiation of isolated strains of enteroviruses associated with the IS.

PROSPECTS FOR FURTHER RESEARCH

Further studies will focus on a comprehensive study of the strains of enteroviruses we have identified, with the identification of those that play an etiopathogenetic role in the emergence and development of the IS. In addition, we consider it expedient to point out the prospects for the prevention of the IS with a vaccine on the basis of the enteroviruses that would be most relevant from the epidemiological and etiopathogenetic point of view.

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ORIGINAL ARTICLE
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SYNDROMAL CHARACTERISTICS OF THE COMBINED COURSE OF CHRONIC PANCREATITIS AND ARTERIAL HYPERTENSION

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ABSTRACT

The aim: To study the clinical course of chronic pancreatitis (CP) in patients with concomitant hypertension.

Materials and methods: A thorough analysis of the results of subjective and clinical and laboratory examinations of 102 patients with CP, who were hospitalized in the therapeutic department of Khust district hospital during 2017-2018 was conducted.

Results: Takingintoaccount that the initial examination of patients was carried out during the period of exacerbation of the disease, all patients presentedwith pain syndrome (100%).The overwhelming majority of patients identified pain as permanent aching discomfort in the abdominal cavity with periodic intensification (n = 41 (40,2%), 18 patients indicated burning pain (17,6%), 30 – cutting (29,4%)), and 13 (12,7%) experienced pain of varying nature.due to the presence of hypertension (GC) 73 (71.6%) patients complained of a headache of a paroxysmal periodic pulsating character, which was most often localized in the occipital-frontal area and was accompanied by dizziness, "flickering of flies" before the eyes, noise in the ears. Also, 14 (13,7%) patients had shortness of breath at moderate physical exercise, lower extremity edema that appeared in the afternoon and disappeared by the morning.In addition, 33 (32,4%) surveyed patients indicated a periodic heartbeat.In 18 (17,7%) patients sporadic dizziness was observed. The level of blood pressure (BP), which was established in the studied patients, corresponded to arterial hypertension of grade 1-2 (154,15 ± 9,24 / 94,53 ± 9,05 mm Hg). In terms of heart rate (HR) in the examined patients, it was 80.26 ± 10.73 beats per minute.

Conclusions: According to the study, the negative impact of concomitant hypertension on the clinical course of chronic pancreatitis was established, namely: hypertension in most cases causes intensification of pain syndrome; pain syndrome in comorbid patients with cronic pancreatitis and arterial hypertension is accompanied by more pronounced manifestations of asthenisation: general weakness, lethargy;dependence of the duration of pain syndrome from the height of blood pressure and the duration of arterial hypertension was found; dyspepsia syndrome is more pronounced, varied and prolonged in patients with high blood pressure; smoking (37,3%), alcohol (19,6%), psycho-emotional overload (6,9%) are common etiologic factors in comorbid patients with chronic pancreatitis and arterial hypertension; BMI results indicate the dominance of excess body mass in most comorbid patients with chronic pancreatitis and arterial hypertension.

KEY WORDS: chronic pancreatitis, arterial hypertension, course

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INTRODUCTION

The significant advances made in modern diagnostics and pharmacotherapy have not solved the problem of chronic pancreatitis (CP), which remains one of the most difficult sections not only of pancreatology, but also of clinical gastroenterology in general. Prevalence, increase in morbidity of chronic pancreatitis, temporary disability and cause of disability is relevant both from social and economic point of view, which is confirmed by the data of epidemiological analysis [1, 2].

Features of clinical course of chronic pancreatitis are often determined by its combination with other diseases in conditions of polymorbidity of the modern patient. Such combinations, as a rule, contribute to greater torpidity of the clinical course of pancreatitis and often increased number of complications [1, 2, 3].

In the studies of T. I. Vyun (2015) it is noted that in most cases (73,7%) chronic pancreatitis preceded the formation of hypertension (GC), in 18,4% of patients arterial hypertension developed earlier; 7,9% of patients could not indicate which pathology occurred first. Clinical course of chronic pancreatitis in patients with hypertension, compared with patients with isolated form of chronic pancreatitis, was characterized by greater severity of abdominal pain syndrome with a tendency to longer exacerbations (3-4 weeks and 10-12 days, respectively).Lower efficacy of pain syndrome therapy was noted: the clinical effect was reached after 9-10 days and 6-7 days, respectively. According to the author's conclusions, the peculiarities of the clinical course of chronic pancreatitis are often determined by its combination with other diseases in the conditions of polymorphism of the modern patient, which is an indication for the expansion of therapy [4, 5].

According to I.G. Pakhomova (2003), the recurrent form of chronic pancreatitis combined with arterial hypertension, compared with the isolated form of the

disease, is characterized by a more severe clinical course: greater severity of abdominal pain syndrome and dyspeptic syndromes with a tendency for longer exacerbations and lower efficacy of basic therapy. At the same time, in patients with an isolated form of CP, the causes of exacerbation are usually errors in the diet (53,13%) and/or alcohol intake (18,75%). On the contrary, in patients with chronic pancreatitis combined with GC, occurrence of these factors is significantly lower (44,4% and 11,15%, respectively), whereas psycho-emotional overload (19,05%) and physical stress have a greater role in the structure of exacerbations(11,15%) [1].

Analysis of the data presented in scientific sources allows us to assert that the features of clinical manifestations of chronic pancreatitis when combined with arterial hypertension have not been sufficiently investigated, which justifies the need for further in-depth studies of the features of the course of chronic pancreatitis in combination with arterial hypertension.

THE AIM

To determine the clinical course of chronic pancreatitis in patients with concomitant arterial hypertension.

MATERIALS AND METHODS

In order to evaluate the clinical course of chronic pancreatitis in combination with arterial hypertension, we conducted a thorough analysis of the results of subjective and clinical and laboratory examinations of 102 patients with chronic pancreatitis, who were hospitalized in the therapeutic department of Khust district hospital during 2017-2018. The average age of the surveyed patients was 51 ± 10 years. The gender distribution revealed a slight predominance of female patients (56%) compared to male patients (44%). The average duration of chronic pancreatic injury ranged from 4 to 10 years.

Diagnosis of chronic pancreatitis was verified on the basis of medical history data, clinical manifestations and results of laboratory and instrumental studies. Assessment of blood pressure was based on the basic requirements of the «Unified Clinical Protocol for Primary, Emergency and Secondary (Specialized) Medical Assistance to Hypertension», approved by the order of the Ministry of Health of Ukraine No. 384 of May 24, 2012, on Clinical Recommendations for Arterial Hypertension of the EuropeanSocietyofHypertension (ESH) and the European Society of Cardiology (EuropeanSocietyofCardiology (ESC) (2013 and 2018).

Pain intensity was assessed according to a 10-point visual-analogue pain scale (J. J. Bonica, 1990). [6].

RESULTS

To evaluate the clinical course of chronic pancreatitis in combination with arterial hypertension, we conducted a thorough analysis of the results of subjective and objective examinations. Taking into account that the initial examination of patients was carried out during the period of exacerbation of the disease, all patients had pain syndrome (100%). Regarding the localization of pain, the majority (71 patients – 69,6%) indicated pain in the epigastric region, 31 patients (30,4%) – in the left hypochondrium. The irradiation of pain was multidirectional.

The overwhelming majority of patients identified pain as permanent aching discomfort in the abdominal cavity with periodic intensification (n = 41 (40,2%), 18 patients indicated burning pain (17,6%), 30 – cutting (29.4%)), and 13 (12.7%) experienced pain of varying nature, in addition, it is important to note that periodic pain was observed in 53 patients (51,9%) and permanent pain in 49 (48,1%). According to the data obtained, it was found that low-intensity pain sensations (within 1 to 3 points) were not reported by any of the patients. Medium intensity pain (4-6 points) was found in the majority of patients with exacerbation of chronic pancreatitis (n = 55; 53,9%), pain sensations of pronounced intensity (7-9 points) were recorded in 47 patients (46,1%.) A pain score of 10 was not found in any of the completed questionnaires.

A statistically significant direct correlation of moderate strength between pain level and α1-AT concentration (r = 0,40; p <0,01) and urine diastase level (r = 0,45; p <0,01) was recorded. Significant positive weak association with blood lipase (r = 0,25; p = 0,01), amylase (r = 0,27; p = 0,006) levels was established. The intensity of pain was also variable (Fig. 1).

According to the data obtained, it was found that patients with poor intensity pain syndrome (within 1 to 3 points) were not observed in the study sample. Medium intensity pain (4-6 points) was found in the majority of patients with exacerbation of chronic pancreatitis (n = 55; 53,9%), pain sensations of pronounced intensity (7-9 points) were recorded in 47 patients (46,1%). A score of 10 points was not detected in any of the completed questionnaires. Identification of statistically significant weak correlation of negative direction between the severity of pain, according to the indicators of the visual-analogue scale, and the duration of chronic pancreatitis (r = -0,27, p = 0,03) was an important element of the study.This information is in line with the opinion of the majority of scientists who confirm the decrease in the intensity of abdominal pain in the process of disease progression, which can be explained by the progressive fibrosis of the pancreatic parenchyma. Pain of low intensity (1-3 points) and pain score of 10 points were not determined in any patient.

In the processof analysis of the dependence of pain on the laboratory parameters studied by us correlation relationships of different strength and direction were established.The dependence of pain on the functional capacity of the pancreas is of interest. Reliable direct correlation of moderate strength between the pain level and α1-AT concentration (r = 0,40, p <0,01) and urine diastase level (r = 0,45, p <0,01) was recorded.Significant positive weak association between bloodlipase (r = 0.25, p = 0,01), amylase (r = 0,27, p = 0,006) levels was found. No statistically

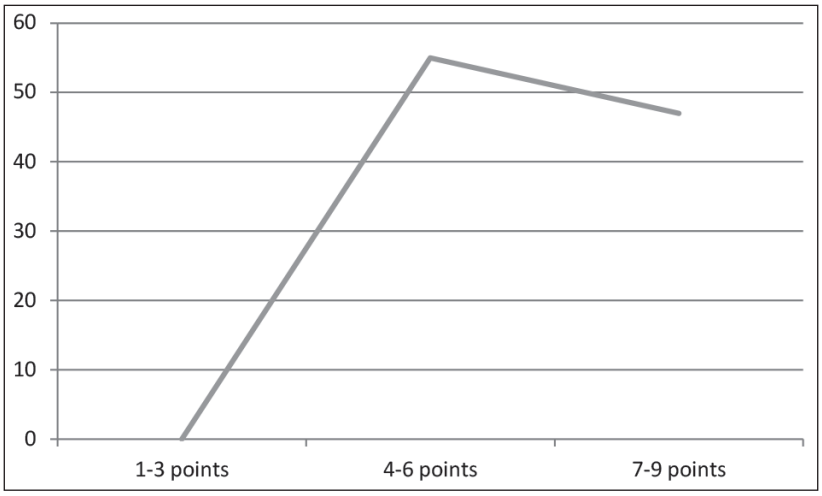

Figure 1. The intensity of pain

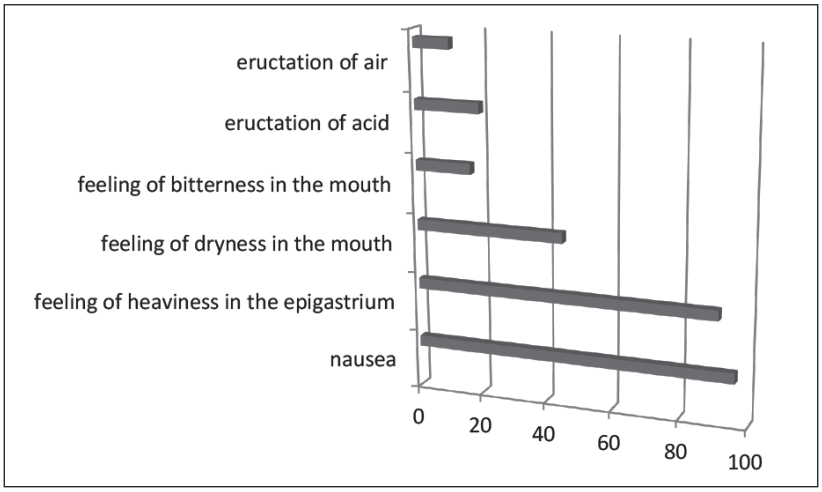

Figure2. Symptoms of the dyspeptic syndrome

significant correlation was found between pain intensity and elastase-1 content in feces. Among the indicators of inflammatory response of the organism a reliable direct modaratestrength correlation between the intensity of pain and the level of leukocytes (r = 0,51, p <0,01), neutrophils (r = 0,42, p <0,01), ESR (r = 0.54, p <0.01) was revealed. A significant positive weak correlation between pain intensity and fibrinogen (r = 0,25, p = 0,01) and cortisol (r = 0,21, p = 0,04) levels was found.

When searching for factors that contributed to the activation of pain, it was found that 57 patients (55,9%) indicated an increase in pain after eating, especially fried, smoked, fatty food (10,7%), freshly baked bakery products, carbonated beverages, semi-finished products.According to the data obtained, 27 patients (26,5%) had sitophobia.In addition, patients noted increased pain in supine position (n = 81; 79,4%) and with change of body position (n = 37; 36,3%).Pain was somewhat relieved in sitting position while leaning forward and being on the left side in a horizontal position with legs lowered to the torso.In addition, to reduce pain, some patients (n = 27; 26,4%) used antispasmodics, analgesics and/or enzyme preparations, but the use drugs mentionedabovedidnot give lasting effect, what eventually forced patients to seek specialized medical care.Patients experienced a predominance of pain syndrome over others, but dyspeptic syndrome and external secretory pancreatic insufficiency syndrome also played a major role in the patients' well-being.Analyzing the relationship between the level of pain and the components of antioxidant protection, we found a significant positive moderate strength correlation with the level of urea (r = 0,44; p <0,01) and bilirubin (r = 0.41; p <0.01).Among the indicators of lipid metabolism, pain was correlated with the concentration of LDL cholesterol (r = 0,20; p = 0,04), HDL cholesterol (r = -0,27; p = 0,01) and Apo B (r = 0,21 ; p = 0,03). The relationship between pain intensity and heart rate was found to be natural (r = 0,27; p = 0,01). Patients experienced a predominance of pain syndrome over others, but dyspeptic syndrome and external secretory pancreatic insufficiency syndrome also played a major role in altering patients' well-being. Dyspeptic syndrome was manifested by the following complaints: nausea, vomiting, feeling of heaviness in the epigastrium, belching, and decreased appetite(Fig. 2).

Nausea, which was indicated by 96 (94,1%) patients appeared to be the leading symptom of the syndrome mentioned above in the surveyed contingent.Nausea

Table I. Blood pressure level and heart rate in the studied patients

Parameters	Blood pressure level (M ± m)
Systolic blood pressure (mm Hg)	154,15 ± 9,24
Diastolic blood pressure (mm Hg)	94,53 ± 9,05
Pulse pressure (mm Hg)	59,62 ± 11,52
Heart rate (beats/minute)	80,26 ± 10,73

Table II. Trophological characteristics of the examined contingent of comorbid patients

BMI (kg/m²)	Absolute number (n=102)	%
< 18,5	2	2
20,0-24,9	29	28,4
25,0-29,9	48	47,1
30,0-34,9	19	18,6
35,0-39,9	3	2,9
> 40	1	1

of paroxysmal periodicity statistically significantly outweighed the constant nausea (n = 74 (72,5%) versus n = 28 (27,5%), p <0,01), appearance of which was facilitated by the consumption of food and alcohol.Some patients (n = 48; 47,1%) had vomiting that did not alleviate the overall condition. The sensation of heaviness in the abdominal cavity was determined in 91 (89,2%) of the surveyed patients. Again, the latter sensations intensified after food consumption, forceing patients to limit the frequency and number of intake.Patients also complained of belching of different character. Most often, patients indicated eructation of acid (n = 20; 19,6%) and air (n = 11; 10,7%). In 17 patients (16,7%), a feeling of bitterness and in 45 (44.1%) dryness in the oral cavity was identified.

As with any inflammatory process, and in the case of exacerbation of chronic pancreatitis in combination with arterial hzpertension, there was a pronounced asthenovegetative syndrome, which manifested as general weakness, rapid fatiguability, drowsiness, dysomnia, increased irritability. General weakness, impaired performance, and rapid fatiguability of varying degrees were present in all patients. Excessive drowsiness was identified in 95 (93,1%) patients, sleep disorders in 43 (42,2%), and increased irritability in 56 (54,9%) patients.

Furthermore, due to the presence of arterial hypertension in73 (71,6%) patients,there were complaints of headache with paroxysmal periodic pulsatile character, which was most often localized in the occipital-frontal area and was accompanied by dizziness, «flickering of flies» in the eyes, noise in the ears. Additionally, 14 (13,7%) patients had shortness of breath at moderate physical exercise and lower extremity edema that appeared in the afternoon and disappeared by the morning. Besides, 33 (32,4%) surveyed patientsindicated periodic heartbeat. In 18 (17,7%) patients sporadic dizziness was observed.

When collecting the history of the disease, it was found that the duration of chronic pancreatitis was 7.00 ± 3.00 years, of arterial hypertension – 5.00 ± 2.00 years. The frequency of exacerbations of chronic pancreatitis varied from 1 to 4 times a year. Thus, the proportion of patients with exacerbation of chronic pancreatitis 1-2 times a year was 67,6% (n = 69), 2-3 times a year – 28,4% (n = 29), 3-4 times a year – 5,9% (n = 6). Consequently, it can be stated that the majority of the surveyed contingent had chronic pancreatitis of moderate and mild severity.

Measurement of blood pressure indicated its rise within 1-2 stage. The obtained results of blood pressure and heart rate are presented in Table I.

The level of blood pressure, which was established in the studied patients, corresponded to arterial hypertension of 1-2 stage (the average value of the investigated parameter was 154,15 ± 9,24 / 94,53 ± 9,05 mm Hg). As for the the heart rate in the examined patients, it was 80,26 ± 10,73 bpm.

The sudden, acute onset of the disease was indicated by 41 patients (40,2%), while the gradual onset with increasing symptoms was recorded in 61 patients (59,8%). According to our observations, the acute onset of abdomenalgia in patients was accompanied by an increase in blood pressure to higher numbers (170-182 / 101-110 mmHg) compared to patients who had a gradual onset and the level of blood pressure ranged from 150-165 / 93-108 mmHg. A quarter of patients used different self-medication methods (before seeking medical help), but the effect of such therapy was insufficient.

Patients with acute onset sought specialized care within 12-24 hours of the onset of the first symptoms of the disease, but patients with less severe symptoms were hospitalized in 7-14 days after the onset of the disease. In 4 (3,9%) patients the complaints mentioned above, started 2-3 months ago, but since the severity of the symptoms was insignificant, they werehopingfor spontaneous recovery. Thus, the leading etiological factor remains the change in the structure of the diet and dietary regimen, but the results of our study confirm that smoking, alcohol, psycho-emo-

tional load play an important role in the emergence and progression of both diseases. The results obtained are in accordance with the data of scientists who study this problem [7, 8, 9].

The evaluation of the trophological status of patients with chronic pancreatitis combined with arterial hypertension was based on the body mass index (BMI) calculated using the Kettle formula (developed by AdolpheQuetelet in 1869): BMI = weight (kg) / height (m2). The results obtained are preseted in Table II.

The results of our studies indicate that the majority of patients (n = 48; 47,1%) were overweight, corresponding to a BMI of 25.0 to 29.9 kg / m^2.

DISCUSSION

In modern research, chronic pancreatitis is considered not only as a combination of local lesions due to the influence of pathochemical inflammatory mechanisms, but as a systemic gastroenterological disease, in the development of which hereditary-constitutional, psychosocial, immunological and psychosomatic mechanisms are involved. It is quite common to have a cohort of patients who have a clear relationship between genesis, disease onset, disease phase, and premorbid features [1,3].The situation is exacerbated by the frequent combination of chronic pancreatitis not only with diseases of the digestive system, but also with cardiovascular diseases, among which, the leading position is occupied by arterial hypertension, since the combination of arterial hypertension and chronic pancreatitis is pathogenically conditioned, primarily through the systemic damage to the vascular system, leading to the development of ischemic effects, activation of the inflammatory component of the disease and metabolic disorders. Features of clinical course of chronic pancreatitis are often determined by its combination with other diseases in the conditions of polymorphism of the modern patient. These combinations, as a rule, contribute to greater torpidity of the clinical course of pancreatitis and often increase the number of complications [3, 5], which is clearly observed in our studies. Assessing the symptom of pancreatic pain, interaction between pain intensity and α1-AT concentration and urine diastase level, as well as less reliable effects of lipase, fibrinogen, and serum cortisol levels may be suggested.

CONCLUSIONS

According to the study data, the negative impact of concomitant hypertension on the clinical course of chronic pancreatitis is established, namely:

- arterial hypertension in most cases causes intensification of pain syndrome;
- pain syndrome in comorbid patients with chronicpancreatitis and arterial hypertension is accompanied by more pronounced manifestations of asthenisation: general weakness, lethargy;
- dependence of the duration of pain syndrome on the height of blood pressure and duration of arterial hypertension is noted;
- dyspepticsyndromeismoreexpressed, variedandprolongedwithhighbloodpressure;
- common etiologic factors in comorbid patients with chronic pancreatitis and arterial hypertensionare smoking (37,3%), alcohol (19,6%), psycho-emotional overload (6,9%);
- BMI results indicate the dominance of excess body mass in most comorbid patients with chronic pancreatitis and arterial hypertension.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

STATISTICAL ANALYSIS OF THE IMPACT OF CLUSTERS ON CARIES PREVALENCE AND INTENSITY IN CHILDREN AGED 6-7 WITH DIFFERENT SOMATIC HEALTH STATUSES

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ABSTRACT

The aim: Determining the influence of cluster factors on the emergence and progression of caries in first- and second-grade children is appropriate in terms of determining the most significant ones.

Materials and methods: The dental status of 73 children, residents of Uzhhorod, who study in the first grades of secondary schools, has been assessed, three groups have been singled (the control group 26 healthy children). The indices of essential micro- and macronutrients in the hair, saliva, serum and urine, as well as anxiety level have been determined and the statistical analysis has been performed.

Results: Decreased magnesium and calcium content in hair, serum, urine and mouth fluid, iodine is absent. Children of the third group have high levels of anxiety (80%), high rates of caries (14,8) and significantly reduced levels of magnesium relative to normal.

Conclusions: In the patients of the main groups, a direct correlation was found between the presence of somatic pathology and the level of anxiety. In 80% of children of the third group (CSPS≥3) the level of anxiety is high In the main group patients, a direct correlation was found between the presence of somatic pathology, the level of anxiety, and the decrease in magnesium; The intensity of caries only interacts with a high level of anxiety (1.00), that is, in children who are in constant stress, the intensity of caries increases.

KEY WORDS: children, younger school age, anxiety level, biogeochemical deficiency of fluorine and iodine, caries prevalence and intensity

Wiad Lek. 2020;73(3):434-440

INTRODUCTION

The high prevalence of permanent tooth caries in children in Ukraine [1-3] raises the problem of prevention as the main one in paediatric dentistry. Due to the fact that teeth are most vulnerable to caries after their eruption [4, 5], it is especially important to find new approaches to creating programs for the prevention of permanent caries, especially at the stage of immature enamel [6].

Since the eruption of the first permanent molars and the state of incomplete mineralization coincides with a period of increased exposure to stress from the adaptation of the child's organism to school, which reduces nonspecific resistance of the body, carrying out preventive measures during this period significantly reduces the likelihood of carious lesions.

The occurrence of caries is largely determined by the environmental conditions in which the child lives, namely biogeochemical deficiency of trace elements (especially fluorine, iodine, calcium, magnesium, etc.). Such natural zones include the Transcarpathian region as a natural environment in which a person lives [1]. The prevalence of permanent tooth decay in 12-year-old children in this natural zone constitutes 91.4 ± 2.3% at an intensity of 11.3 ± 0.1 [7, 8].

THE AIM

To establish the correlation between different parameters of micro- and macro elements content in saliva, urine and hair, anxiety level and caries incidence rates in children aged 6-7 with different somatic health status.

MATERIALS AND METHODS

The dental status of 73 children, residents of Uzhhorod, who study in the first grades of secondary schools, was assessed. Three groups were singled out depending on the coefficient of severity of the general somatic pathology. The control

Table I. Indicators of anxiety levels, microelements level in oral fluid, hair, serum and urine in children with different prevalence percentage of caries

Clinical groups Indicators	Group 1 (n = 28)	Group 2 (n = 25)	Group 3 (n = 20)	Control group (n=26)
Anxiety level				
High anxiety level AI>50%,	8,34±0,48*	39,25±1,82*	84,20±2,34*	-
Medium anxiety level from 20> AI< 50%	26,80±0,95*	45,65±1,02*	13,50±0,20*	4,70±0, 01
Low anxiety level AI<20%	64,86±1,76	15,10±0,45*	2,30±0,12*	95,30±1,35
The level of microelements in the hair				
				norm
Ca (Calcium)	310 ±16,02	250±15,12	140±16,30* º	300,00–700,00
Zn (Zinc)	125±10,03	110±8,01*	86±7,37* º	120,00–200,00
K (Kalium, potassium)	100±8,56	140±7,45	170±8,21	70,00–170,00
Mg (Magnesium)	24,00±0,21	20,00±0,48*	14,00±0,16* º	25,00-50,00
I (Iodine)	0,1*	0,0*	0,0* º	0,40 –4,00
Fe (Ferrum, Iron)	23,0±0,61	27,0±0,56	33,0±1,45	6,00–35,00
Cu (Cuprum)	7,20±0,08	5,60±0,01*	4,30±0,08* º	9,00–30,00
Se (Selenium)	0,20±0,01*	0,16±0,08*	0,13±0,04* º	0,30–1,20
Mn (Manganese)	0,27±0,01*	0,17±0,07*	0,0±0,0* º	0,50–2,00
Cr (Chrome)	0,27±0,05*	0,18±0,03*	0,12±0,05* º	0,50–5,00
The level of microelements in the serum				
Mg, mmol/L	1,01±0,1	0,45±0,05*	0,31±0,05*	0,70-1,15
Ca, mmol/L	2,36±0,02	2,01±0,01	1,84±0,02*	2,25-2,60
Ph, mmol/L	1,60 ±0,11	1,30±0,13	1,10±0,09*	1,45-1,77
Zn, mcmol/L	16,21±1,34	9,71±0,54	8,95±0,46	9,18-18,36
Fe, mcmol/L	16,20±1,27	11,01±1,61	9,56±1,42	10,74-30,08
Cu, mcmol/L	20,53±2,19	10,12±1,32	9,27±1,21	11,02-24,39
Se, mcmol/L	1,62±0,04	0,45±0,05	0,38±0,13*	0,58-1,77
Excretion level of microelements in urine				
Mg in urine, mmol/L	0,84±0,01*	0,76±0,03*	0,73±0,03*	0,97±0,03
Ca in urine, mg / day	188,2±18,3	195,6±14,1	201,7±16,5	183,2±23,1
Ph, in urine g / day	0,94±0,10 *	1,03±0,16*	1,37±0,15*	0,88±0,10
The level of microelements in the oral fluid				
Mg mmol/L	0,06±0,01	0,03±0,01	0,01±0,01	0,08-0,53
Ca tot., mmol/L	1,31±0,12	2,41±0,03	2,89±0,04	1,28±0,12
Ca ion., mmol/L	1,08±0,05	1,39±0,09	1,98±0,09*	0,94±0,05
P, mmol/L	5,91±0,31	5,13±0,11	5,56±0,21	6,09±0,37
Alkaline phosphatase, activity unit	26,61±1,02	44,04±0,81*	51,43±1,98*	21,48±1,02
Caries prevalence and intensity				
Caries prevalence	88,6±5,60*	93,2±3,80*	96,4±1,98*	65,3±2,89
Caries intensity (cf+CFE)	5,3±0,20	8,6±0,23*	14,8±0,18*	4,1±0,23

Note: * - significance of differences between indicators of a conditional norm or control group ($p < 0.05$).
º - significance of differences between the first and third groups ($p < 0.05$).

comparison group consisted of 26 children of the same age, residents of Uzhhorod free from caries, i.e. healthy children.

The examination and treatment of children were carried out in the dental offices of the clinical base of the Department of Paediatric Dentistry at the Higher Educational Institution "Uzhhorod National University" in Uzhhorod Municipal Children's Outpatient Clinic. All the children attended secondary schools. The work began after the children's parents or guardians had given their informed consent to participate in the study.

In the surveyed children in the clinical groups, the following indicators were analyzed: caries intensity (cf + CFE index)

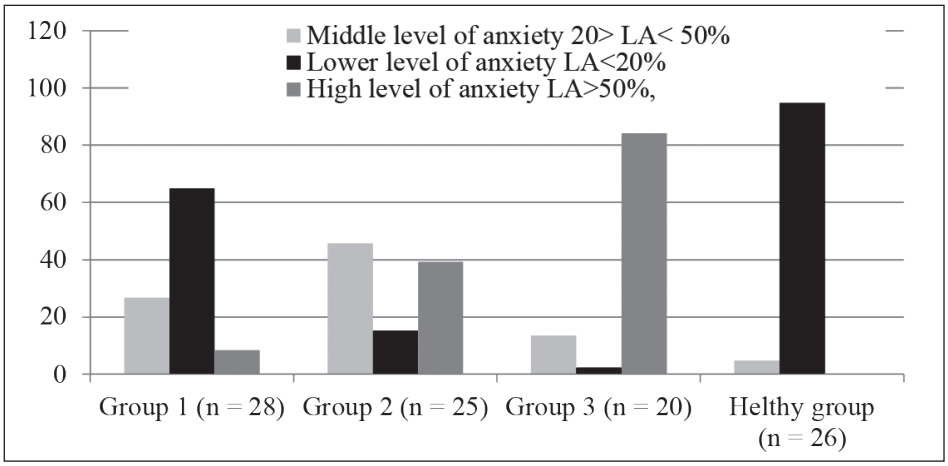

Fig. 1. Childrens anxiety in different groups .

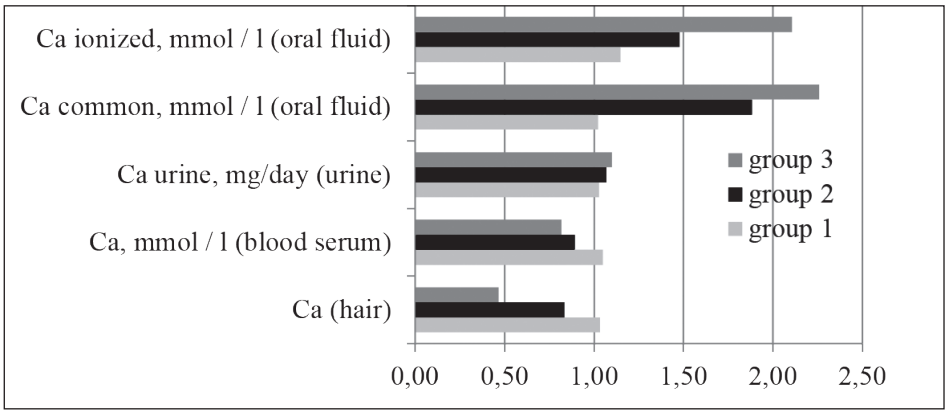

Figure. 2. The relatively proportion of calcium in the body of children to normal level.

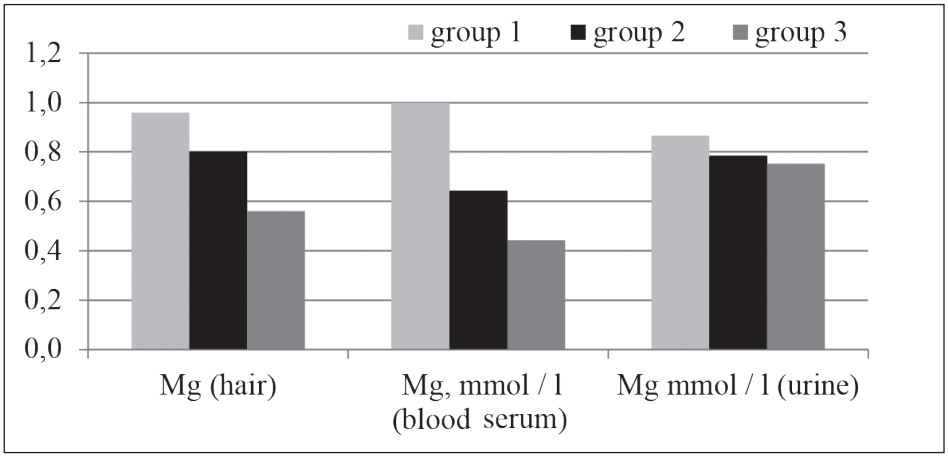

Fig. 3. The relative part of magnesium in children to normal level.

[8], the anxiety level in children according to the test developed by the American psychologists R. Temml, M. Dorca and V. Amen [9]. The indicators of essential elements in the surveyed children in clinical groups were determined as magnesium and calcium levels in the serum, hair, oral fluid, in urinary excretion levels of calcium and magnesium [8].

The statistical analysis of the studied indicators was conducted in the children in three groups; healthy children were adopted as the control group according to which the calculations were made. Statistical processing of the results was performed using the correlation (according to Pearson (r)) and cluster (Euclidian distance method) analyses. All calculations were performed on the PC using licensed "MS Excel 7" software for the "Windows" operating system and the standard software package "STATISTICA" v. 6.0.

RESULTS

To determine the relationship between caries intensity, stress level, and essential element indices in the surveyed children in the clinical groups, the following indicators were analyzed: anxiety level, caries intensity (cf + CFE index), magnesium and calcium levels in the serum, hair, oral fluid, urinary excretion level of calcium and magnesium. (Tabl.I)

Figure 1 shows the distribution of anxiety in the studied groups of children.

It was found that 95 % of healthy children in the control group had low levels of anxiety; 60% of children in the first main group had low levels of anxiety and 20% had medium levels of anxiety. 50% of the children in the second group had a medium level and 40% had a high level of anxiety;

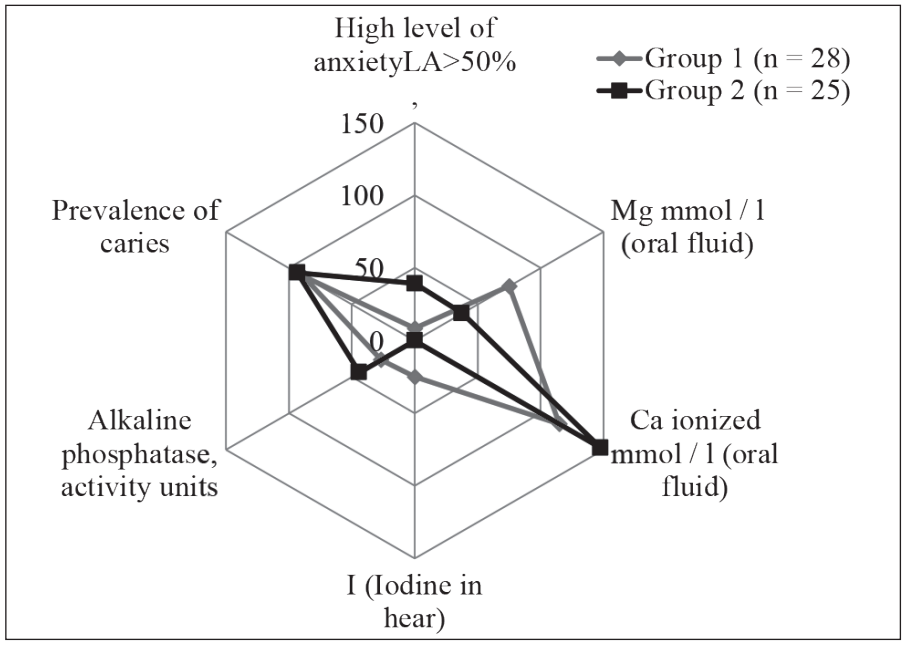

Fig. 4. Indicators of Mg, Ca ion., I, alkaline phosphatase content in two groups of children with high caries prevalence and anxiety level less than 50% (low and medium levels)

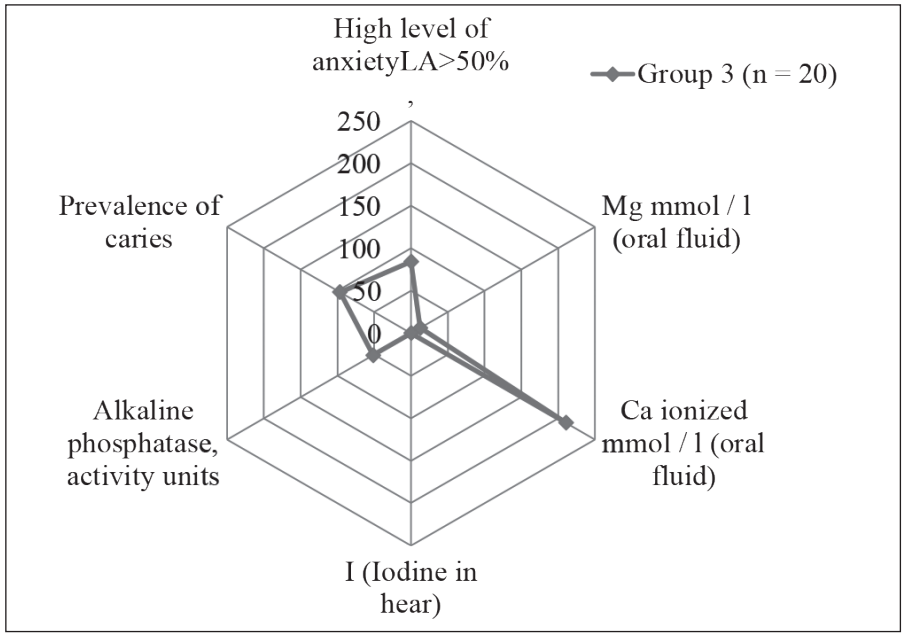

Fig. 5. Indicators of Mg, Ca ion., I, alkaline phosphatase content in third group of children with high caries prevalence and high anxiety level

80% of the children in the third group were diagnosed with high levels of anxiety.

Figures 2 and 3 analyze the indicators of calcium and magnesium in the children in the main groups with regard to the minimum norm values.

In the second group, the indicators of ionized and total calcium in the oral fluid are significantly higher than the norm, in the third group, these indicators are twice higher than the norm. The calcium indicator in urine is slightly higher in the second and third groups. Calcium levels in the serum and hair are consistent with the norm in children in the first group, while these indicators in the second and third groups are below the norm. The content of magnesium in the serum in the children of the first group is consistent with the norm, in other patients, magnesium in the hair, serum and urine is understated and ranges from 50 to 75%.

The ratios of caries prevalence, magnesium and calcium ionized content in the oral fluid, iodine in the hair and alkaline phosphatase activity units in two groups of children with low and medium anxiety levels are analyzed below (Figure 4).

Figure 4 shows the correlation between Mg, Ca_{ion}., I, and alkaline phosphatase levels in the children of the first and second clinical groups that do not exhibit high levels of anxiety (i.e., the anxiety rate constitutes less than 50%). The prevalence of caries in both groups is high and reaches 100%. Compared to the first group, in the second group of children, a high level of anxiety (medium), an increased rate of calcium ionized and a reliably increased level of alkaline phosphatase in the oral fluid are observed. The increase in the indicator of the latter in the children of the second group compared to the healthy children and the children of the first group is associated with an increase in free calcium in the oral fluid.

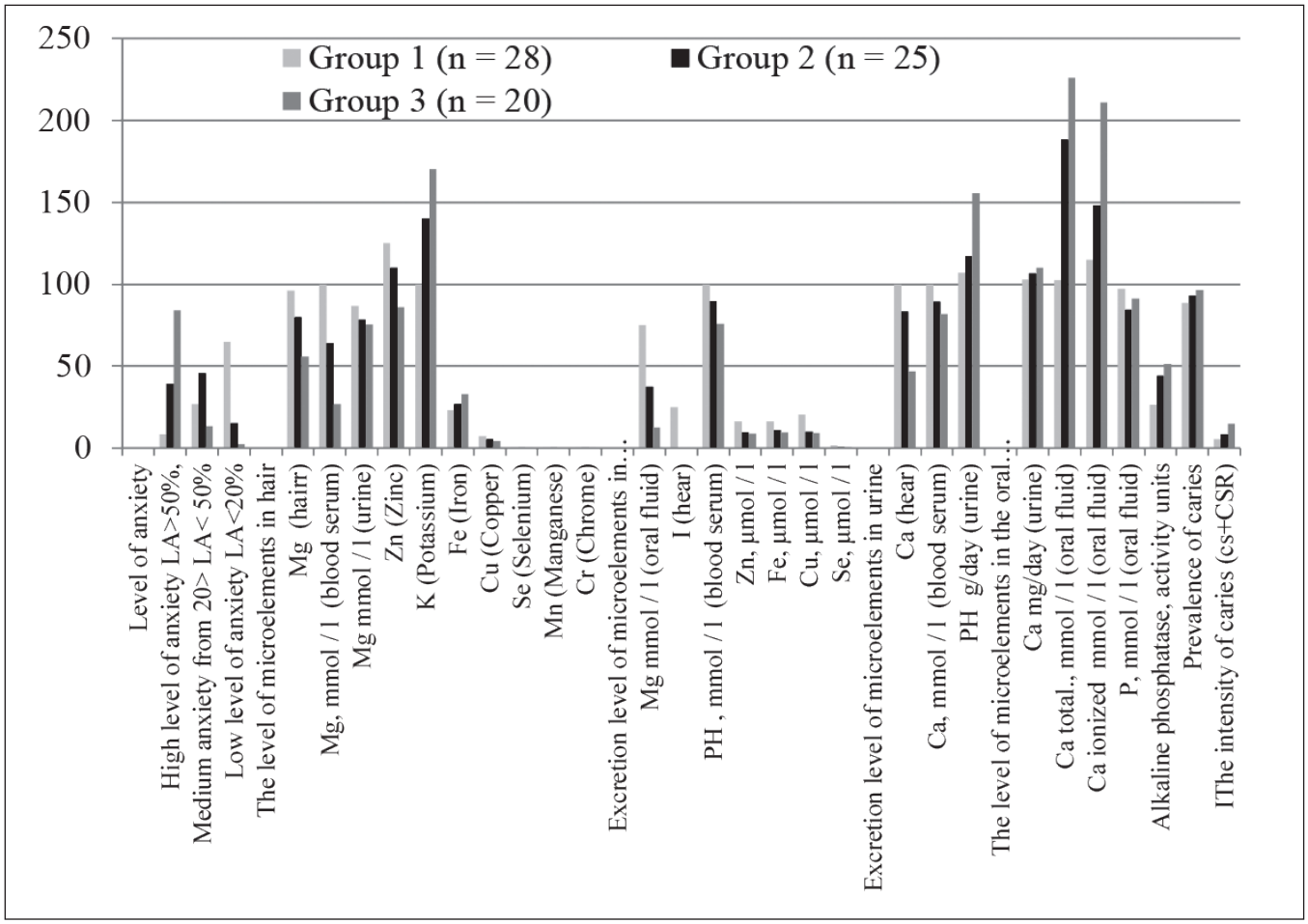

Fig. 6. The main clusters as a factors for the prevalence and intensity of caries

Table II. Correlation dependence of the researched indicators.

	High anxiety level AI>50%,	Low anxiety level AI<20%	Caries preval.	Alkal. phosphatase	Ca tot., in oral fluid	Ca ion., in oral fluid	P[H] of urine g/day
Low anxiety level AI<20%	–0,91	1,00	–0,90	**–0,99**	**–0,99**	–0,94	–0,90
Zn (Zinc)	**–0,97**	0,81	–0,57	–0,89	–0,77	–0,75	–0,64
K (Kalium)	**0,96**	**–0,99**	0,89	**0,99**	**0,98**	**0,96**	0,91
Fe (Ferrum)	0,83	–0,93	**0,99**	0,88	**0,97**	0,99	1,00
Cu (Cuprum)	–0,94	**0,99**	–0,92	**–0,97**	**–0,99**	**–0,98**	–0,94
Se (Selenium)	–0,84	**0,96**	**–0,99**	–0,91	**–0,99**	**–0,99**	**–0,99**
Mn (Manganese)	–0,92	**0,96**	–0,94	–0,94	**–0,98**	**–1,00**	**–0,97**
Cr (Chrome)	–0,83	0,95	**–0,99**	–0,90	**–0,99**	**–0,99**	**–0,99**
Ca (Calcium) in hair	–0,72	0,87	**–1,00**	–0,80	–0,93	–0,96	–0,99
P_h of urine g/day	0,80	–0,90	**0,99**	0,84	**0,95**	**0,99**	1,00
Ca tot., mmol/L in oral fluid	0,89	–0,99	**0,95**	**0,96**	1,00	**0,98**	**0,95**
Ca ion., mmol/L in oral fluid	0,88	–0,94	**0,97**	**0,91**	**0,98**	1,00	**0,99**
Caries prevalence	0,74	–0,90	1,00	0,83	**0,95**	**0,97**	**0,99**
Cariws intensity (cf+CFE)	**1,00**	–0,89	0,73	0,94	0,88	0,88	0,80

The levels of magnesium in the oral fluid and iodine in the hair in the second group (with medium levels of anxiety) are significantly lower, compared to those in the first group and the healthy children, who have a prevailing low level of anxiety. Thus, with an increase in anxiety levels and a decrease in magnesium content, iodine concentration is reduced, which is also observed in the children in the second group.

The correlation of the above-mentioned indicators in the third group of children with high levels of anxiety is analyzed below (Fig. 5).

In the third group, high anxiety is registered in 80% and the prevalence of caries is high. The level of calcium ionized in the oral fluid is much higher in comparison with all groups of the studied children, although the level of alkaline phosphatase remained at the same level as in the second group. The amount of magnesium is critically low and iodine is not found at all.

In order to determine the dependence of the levels of anxiety, caries prevalence and intensity and the examined elements in the oral fluid, blood, urine and hair, further correlation analysis was carried out. Table 2 shows the results of correlative dependencies. The bold font shows the figures that showed a reliable correlation with $p < 0,05$. The presented list includes only those indicators against which reliable correlation can be observed in more than four cases. (Tabl.II)

Figure 6 presents the most important indicators according to the studies conducted, with the help of which it is reasonable to analyze caries prevalence and intensity, taking into account different levels of anxiety in the children of the three analyzed groups.

DISCUSSION

Magnesium ions are involved in all life processes that occur in the body without exception, and facilitate their course. Magnesium deficiency inhibits these processes. It is a bioelement that effectively affects everything that happens in cells. Rapid fatigability, difficulty concentrating, sensitivity to changes in the weather, cold, changes in humidity, which causes pain in the teeth, gums, joints, muscles, as well as fears, uncontrolled irritability, unwillingness to do several things at once – these are just some of the symptoms that indicate magnesium deficiency in the body. Therefore, the insufficient level of this element in the children of the second and third groups, indicates a decrease in the resistance of their organisms to negative factors, and may indicate an increased level of anxiety.

In order to determine the dependence of the levels of anxiety, caries prevalence and intensity and the studied elements in the oral fluid, blood, urine and hair, further correlation analysis was carried out.

The conducted correlation analysis found the correlation indicators of magnesium in the body, which are not shown in the table, since they reliably correlate only with each other. Mg (Magnesium) in hair correlates only significantly with Mg, mmol/L in the serum (0,98), and the latter correlates only with Mg mmol/L in the oral fluid (0,99). Caries intensity correlates only with high levels of anxiety (1,00) and K (0,96), showing an inverse interdependence with Zn (0,97).

Iodine manifests significant correlations in four cases: Zn (1,0), Fe (0,98), Cu (0,99), Se (0,99). With Mg mmol /L in the oral fluid it shows a correlation with $p> 0,05$ (0,88). Therefore, the amounts of magnesium and iodine in the children's organisms do not show correlatively reliable dependence. This may mean that the amounts of elements in the body are not directly interdependent, and they may be related through certain processes that occur in the body during the transportation, transformation or excretion of these elements.

High anxiety level AI> 50% gave a significant correlation with K (0,96), Zn (–0,97), alkaline phosphatase (0,96) (see Table 2). That is, a high level of anxiety in a child is accompanied by an increase in the amount of potassium and a decrease in the amount of zinc in the body. There is also a correlation of high anxiety at $p> 0,05$ with Cu (–0,94), Se (–0,84), Mn (–0,92), Cr (–0,83), which may indicate the presence of an indirect connection, for example, due to the quality of intermediate processes that occur in the organisms of the children in the studied groups.

Low anxiety level AI <20% with K correlates in the opposite direction (–0,99), correlates significantly with Cu (0,99), Se (0,96), Mn (0,96), Cr (0,95) in direct relation, as opposed to the correlation of these indicators with high levels of anxiety. A reliable negative correlation of low levels of anxiety was also found with Ca, mmol/L in oral fluid (-0,99) and alkaline phosphatase, activity unit (-0,99). An unreliable correlation was found with Ca ion., mmol/L in the oral fluid (0,96), caries prevalence (-0,90) and caries intensity (cf + CFE) (-0,89).

Caries prevalence reliably correlates with Fe (0,99), pH of urine g / day (0,99), Ca tot., mmol / L in the oral fluid (0,95), Ca ion., mmol /L in the oral fluid (0.97). Negative dependence is observed with Se (-0,99) Ca in the hair (-1,0).

Thus, the higher the prevalence of tooth decay, the lower the calcium level in the hair and the higher the calcium level in the oral fluid (Ca ion. and Ca in the hair shows (0,98) correlation, Ca ion. and the prevalence of caries gives (0,97), Ca tot. and caries prevalence manifest (0,95) positive correlation, at an intensity of caries and Ca tot. and Ca ion. the correlation constitutes (0,88), however, at $p> 0,05$). Ph of urine g / day also exhibits a dependence with the prevalence of caries (0,99), as well as with calcium Ca ion. (0,99) and Ca total (0,95). Accordingly, the dependence of alkaline phosphatase with the content of total and ionized calcium in the oral fluid (0,96 and 0,91 correspondingly), is logical. It should also be noted that there is an inverse dependence of alkaline phosphatase and the low level of anxiety in children, that is, the indicator of alkaline phosphatase is low at the low anxiety level, and it grows at the high anxiety level with the probability of correlation (0,99).

Figure 6 shows that from the first to the third groups in the direction of increase proceed the following indicators: the level of anxiety, the amount of calcium in urine per day; the amount of ionized and total calcium in the oral fluid varies significantly between groups; the indicators of alkaline phosphatase, intensity and prevalence of caries are also increasing.

In the direction of decrease from the first group of investigated children to the third one, the following indicators can be seen in the figure: magnesium content in the hair, blood serum, urine and oral fluid. Iodine is absent in the hair in the second and third groups. Calcium indicators in the hair and blood serum also decrease.

The children in the third group have a high level of anxiety (80%), high intensity of caries (14.8) and significantly reduced magnesium levels relative to normal. And only

these children have calcium indices slightly deviated from the norm, while in others they are much less than normal.

CONCLUSIONS

1. In patients of the main groups a direct correlation between the presence of somatic pathology (the coefficient of somatic pathology severity) and the level of anxiety was established, in 80% of children of the third group (CSPS≥3) the level of anxiety was high, low level of anxiety prevailed in the first group.
2. There is a high prevalence of caries in all groups. Children with high anxiety levels have decreased levels of magnesium and iodine in the body and increased amounts of ionized and total calcium in the oral fluid. Moreover, the alkaline phosphatase index correlates significantly with the prevalence of caries (0,96) and reaches 44–52 units of activity in the 3rd group of children.
3. The indicators of magnesium content in the studied groups of children show reliable correlation dependencies only among themselves. However, in general, the absolute values of the investigated indicators depend on the level of anxiety and the decrease in magnesium in the body is obvious.
4. The intensity of caries interacts only with a high level of anxiety (1,00), i.e. in children who are in constant stress, the intensity of caries increases.
5. The indicators of total and ionized calcium in the oral fluid and the level of anxiety in the patient may be the first to indicate the prevalence of caries, its intensity, the possible deficiency of all elements in the body that form the clusters.
6. In children of the third group, a high level of anxiety, high intensity of caries and significantly reduced rates of magnesium content relative to the norm are observed; only children in this group have calcium rates unreasonably below normal (p> 0,05).

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ORIGINAL ARTICLE
PRACA ORYGINALNA

COMPARISON OF THE EFFECTS OF TOFACITINIB AND ADALIMUMAB ON TRANSCOLONOSCOPIC PH AND CALPROTECTIN LEVELS IN PATIENTS WITH ULCERATIVE COLITIS

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ABSTRACT

The aim: To investigate the transcolonoscopic pH-metry and calprotectin in patients with ulcerative colitis.

Materials and methods: the research included 110 patients both male and female between the ages of 18 to 75 years old, who were treated for UC of medium and severe activity, in active phase. All patients were divided into 3 groups. The first group received standard therapy (ST; n=50), the second group received adalimumab (ADA; n=32), and the third group was treated with tofacitinib (TOF; n=28). The control group consisted of healthy individuals between the ages of 18 and 65 years old.

Results: UC patients had lower pH levels in all sections of the large intestine, compared to the control group ($p<0,05$). Calprotectin level is a better predictor of the course of the disease.

Conclusions: Tofacitinib, compared to adalimumab and budesonide, has better influence on clinical, endoscopic and laboratory parameters of UC.

KEY WORDS: ulcerative colitis, calprotectin, pH-metry

Wiad Lek. 2020;73(3):441-443

INTRODUCTION

Ulcerative colitis (UC) is a multifactorial disease, which manifests in a chronic, recurrent bowel inflammation. Its course is undulating and continuous. The etiology is still unclear, but it was proven, that there is a complex interaction between genetic, microbiologic and external factors. [1, 2]

Endoscopic and morphologic methods are the main diagnostic methods in UC, which enable us to assess the activity of the process and its prevalence. But even these methods aren't accurate enough. That's why physicians are in dire need of a method which would be accurate, simple and available, so they may quickly and precisely assess the state of the digestive system [3,4]. Latest researches have a goal of developing new diagnostic and treatment methods for chronic IBD, to achieve a clinical and endoscopic remission, prevent relapse and increase the quality of life.

Problems with pH measurement in the large intestine have great theoretic and clinical value, because pH levels facilitates the diagnostic process and the course of treatment. In UC most of the mucous membrane is intact. Absorption and secretion are altered, as well as electrolite and water exchange between colonocytes and contents of the intestine. Colonocytes secrete less bicarbonate, which results in lesser absorption of acidic compounds. In addition, colitis increases the intensity of fermentation processes, which is also accompanied by the accumulation of a large number of short-chain fatty acids in the gut that acidify the environment. Also, UC increases fermentation, leading to accumulation of short-chain fatty acids, which lower the pH even further. Lactic acid production is increased in severe UC. [5,6] Non the less, large intestine pH mesurment is a difficult process in most clinical situations, because of its complexity, bulkiness, inaccuracy and limitation.

Nowadays the easiest and most informative method for measuring large intestine pH is via pH-probe during fibrocolonoscopy. The potential of diagnostic laboratories has increased in recent years, due to technological progress. They can detect biomarkers of inflammation not only in serum, but in feces as well.

Calprotectin is one of the markers of neutrophilic inflammation and an indicator of the intensity of the inflammatory process of intestinal inflammation in diseases of the digestive tract. It is a calcium binding protein, with antibacterial and antifungal activity, induces apoptosis in malignant and benign cells. This protein is stable, remains in feces at room temperature up to 7 days, whih makes it an ideal diagnostic marker. [7,8]

First research on calprotectin was conducted in Norway in 1997 p. A.G. Roseth et al. Levels of fecal calprotectin were assessed in patients with active UC, inactive UC and in a control group. The levels were 68, 11.5 and 6 mg/l respectively. The conclusion was made, that fecal calprotectin may be used as a marker of disease activity. [9]

Fecal calprotectin is a marker of intestinal inflammation and provides the opportunity to noninvasively differentiate between irritated bowel syndrome and IBD, to monitor the course of the disease/treatment of UC. It's a potential

Table I. pH levels in different sections of the large intestine before and after treatment (M±m)

Large intestine secrion		ST	ADA	TOF	Control
Rectum	Before treatment	7.02±0.16	7.04±0.13	7.05±0.15	7.62±0.23
	After treatment	7.02±0.14	7.06±0.12	7.18±0.12	
Sigmoid	Before treatment	6.84±0.07	6.92±0.08	6.94±0.08	7.64±0.19
	After treatment	6.88±0.08	6.97±0.07	7.27±0.10	
Transverse	Before treatment	7.10±0.24	7.10±0.22	7.03±0.17	7.44±0.17
	After treatment	7.11±0.04	7.20±0.06	7.22±0.04	
Cecum	Before treatment	7.01±0.06	6.98±0.04	7.01±0.04	7.26±0.16
	After treatment	7.01±0.05	7.01±0.04	7.11±0.04	

p<0,05 for all groups, compared to healthy patients and during treatment

screening marker for colorectal neoplastic processes. This marker provides a diagnostic result without using radiologic or endoscopic tests.

The main benefit of fecal calprotectin is its diagnostic value: low levels indicate an absence of organic disease of the intestine, while in active UC the levels are much higher. [10]

THE AIM

To investigate the transcolonoscopic pH-metry and calprotectin in patients with ulcerative colitis.

MATERIALS AND METHODS

The research included 110 patients, which were treated for medium and severe UC in active phase in CE "Uzhhorod regional hospital" in 2017-2018. The diagnosis UC was based on clinical, endoscopic and histological findings. The activity of the disease was assessed using the index of clinical activity and the MAYO score (Shroeder KV, 1987). At time of inclusion, the duration of UC was not less than 6 months and the index of pathologic activity was between 6 and 12. Both male and female patients, between the ages of 18 to 75 years old were included. Data was gathered from patients' history records, ambulatory medical records and anamnesis morbi. Patients were divided into 3 groups depending on the treatment received. The first group received standard therapy (ST; n=50), the second group received adalimumab with a starting dose of 160 mg and 80 mg on the second week of treatment, and a *supporting dose* – 40 mg weekly(ADA; n=32), and the third group was treated with tofacitinib 10 mg b.i.d. (TOF; n=28). The control group consisted of 30 healthy individuals between the ages of 18 and 65 years old.

Clinical, instrumental and laboratory tests were performed all of patients. Also calprotectin and pH levels were measured. Transcolonoscopic pH-metry was performed.

Statistical analysis was conducted using a variational-statistical method. Mean values (M), their standard deviation (m) and confidence intervals were calculated. The probability of differences was estimated by Student's t-test for dependent and independent samples.

RESULTS

Prior to treatment the endoscopic activity index (EAI) was almost identical and corresponded with medium severity of UC (9.22±0,40). Statistically significant improvement of the EAI was observed in all four groups after treatment.

Measurement of pH levels near the mucous membrane of the large intestine in healthy people, showed a tendency toward alkalosis, which increased in the distant segments (7.24±0.16; 7.42±0.16; 7.62±0.17; 7.62±0.22 in the cecum, transverse colon, sigmoid colon and rectum respectively). Such differences in pH levels throughout the large intestine may be associated with the microbe homeostasis, volatile fatty acid production and the difference in absorption rate in the cecum and rectum, which is much higher in proximal regions of the intestine.

Dynamic changes of transcolonoscopic pH levels before treatment in ST, ADA and TOF groups showed a statistically significant decrase of those levels in all sections of the large intestine, compared to the control group ($p<0.05$). This tendency remained after treatment as well ($p<0.05$). This means, that the treatment led to improvement of pH levels in UC patients, but didn't normalize them, as in healthy patients. (Table 1).

Data analysis showed statistically significant differences in pH levels were found in the sigmoid colon , transverse colon and cecum, when comparing groups before and after treatment (TOF) ($p<0.05$).

Fecal calprotectin levels were high in all groups prior to treatment, mean concentration was 466.4±35.6 μg/g(norm 50 μg /g).

In the I group, which received standard therapy, calprotectin dropped to optimal levels in 26.0% (n=13) of patients 122.4±9.1 μg/g, in the II group – in 56.3% (n=18) of patients mean FC level was 102.6±10.2 μg /g, and in the III group – most of the patients – 71.4% (n=20) – mean FC level was 96.2±9.4 μg /g. FC levels remained high in all other patients: in the I group – 74.0% (n=37) mean FC levels was 252.4±12.6 μg /g, in the II group – 43.7% (n=14) mean levels were 212±8.4 μg /g and in the III group – 28.6 % (n=8) it was the lowest – 162±9.2 μg /g. All the patients that with high levels of calprotectin, had a relapse of UC during the next 6 months.

From this data we may conclude, that FC levels a high sensitivity rate for monitoring the course of UC.

Despite the fact, that FC levels didn't return to normal in any of the groups (<50 μg /g), the best results were exhibited by the III group of patients, which received tofacitinib.

DISCUSION

In this research we investigated the pH levels near the wall of the large intestine and calprotectin levels in UC patients, which received budesonide, adalimumab and tofacitinib. These tests allow us to better determine the state of the intestine and to monitor the course course and treatment of ulcerative colitis.

Coincidentally, the patients that had high levels of calprotectin after treatment, had a relapse of UC in the duration of 6 months. This leads to the conclusion that calprotectin is a more sensitive marker of ulcerative colitis.

Tofacitinib – janus kinase inhibitor, had a greater treatment effect, compared to budesonide and adalimumab.

CONCLUSIONS

1. Tofacitinib, compared to budesonide and adalimumab, has greater effect on clinical, endoscopic and laboratory signs of UC.
2. In UC patients in the end of the treatment, near wall pH levels in the large intestine improved slightly. The best improvement was in the III group (TOF).
3. Calprotectin – is a more sensitive marker for diagnosis, differential diagnosis and monitoring the course of ulcerative colitis.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

A RESPONSIBLE PATIENT: FROM THEORY TO PRACTICE ON A MODEL OF A PATIENT WITH BRONCHIAL ASTHMA

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ABSTRACT

Introduction: The medicine of today is focused on personalized aid to a patient, which involves partnership and mutual responsibility between the patient and the doctor. In our reality, the patient believes that the responsibility for the treatment and outcome depends on the doctor in most cases.

The aim: Induce the patient to develop responsibility for the course of asthma against the background of EBW or obesity and introducing this program into the practice of family physicians.

Materials and methods: By design, the study included 75 patients with asthma with varying degrees of severity, persistent course in the remission stage against the background of EBW or obesity. According to the randomization method, the patients were divided into 3 groups: the main group – 30 patients (the use of medication), the comparison group – 30 patients (training in the "Asthma School"), the 3rd group was controls of 15 patients (basic therapy).

Results: According to the anthropometric study, 75 patients enrolled in the study were found to have EBW or obesity and the mean BMI was 31.67 ± 0.53 kg / m^2. It was also found that 20 (66.67%) patients in the main group and 21 (70.00%) patients in the comparison group did not regularly use basic therapy and cancelled or changed the drugs without permission. After the training, 45 patients (76.66%) of the total patients of the main group and the comparison group began to perform respiratory movements with the inhalation device correctly. patients in the main group reduced body weight on an average by 4.5 kg, and BMI from 32.00 kg / m^2 changed to 29.7 kg / m^2 ($p < 0.05$), although they did not reach 25.0 kg / m^2 of the target level because the follow-up period was not long.

Conclusion: Only patients in the main group had significant positive changes in asthma control, the patients in the comparison group also had positive dynamics, but it did not show statistically significant differences. The doctor should be interested in the fact that at the first and subsequent visits regarding the disease, in our case it is bronchial asthma, the patient will feel responsible for the disease. Therefore, when communicating with the patient, the physician should focus on this, provide the most complete answers to the patient's questions, provide educational literature and, if necessary, diaries of self-control.

KEY WORDS: bronchial asthma, excessive body weight, obesity, responsible patient

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INTRODUCTION

The medicine of today is focused on personalized aid to a patient, which involves partnership and mutual responsibility between the patient and the doctor. In our reality, the patient believes that the responsibility for the treatment and outcome depends on the doctor in most cases. This postulate is not objective and does not help to achieve a complete relationship between the patient and the doctor, which in turn impairs the outcome of treatment.

Referring to the urgency of this issue, we can say that when searching for papers and publications on this topic in Ukrainian or Russian, we only received a reference to the Criminal Code, which contains articles on criminal responsibility of doctors or patients. However, there are no data indicating how to encourage the patient to cooperate with the further development of the patient's responsibility for his or her state of health, treatment, etc.

As for the English-language sources, we have a number of publications that do not make its aim to improve our understanding of the patient's responsibility. Thus, one of the first publications on this topic refers to 2003 [1], which states that the future of medicine should be aimed at the formation of an "independent" patient who will have sufficient knowledge about the disease, methods of treatment, etc that he will receive from the doctor and will follow these recommendations.

Another study, conducted at 77 hospitals in China, with the character of a national cross-sectional study with a certified cluster sample of patients, showed a multivariate regression analysis in 2014-2015 where the strongest predictor of patients' responsibility to healthcare providers was patient's trust and then the level of the patient's education. Acquaintances with healthcare providers and the frequency of hospitalizations in the past have had a reciprocal relationship with patients' sense of responsibility to healthcare providers [2].

It is also important to keep in mind that there is a problem with the use of Internet resources, namely that people are increasingly using the Internet resources to access the healthcare industry and related information, which is often incorrect or misleading the internet user [3,4].

Some scientists state that patients should have responsibilities that include respect and gratitude to the doctor [5,6,7,8]. However, empirical evidence indicates that patients have limited feelings of responsibility to physicians.

At the same time, it is necessary to understand which patients are most important to have a responsible attitude to themselves, their disease and treatment. In our opinion, these are patients with chronic and/or comorbid pathology. According to modern statistics, bronchial asthma (BA) is one of the leading problems in primary care medicine [9,10]. It is not news that these patients should constantly follow maintenance therapy, which includes basic treatment and a specific lifestyle. However, it has been found that patients with asthma who have comorbid pathology in the form of excess body weight (EBW) or obesity have a more severe disease course and have a low control of the disease course [11,12,13].

THE AIM

The aim encouraging the patient to develop responsibility for the course of asthma against the background of EBW or obesity and introducing this program into the practice of family physicians.

MATERIALS AND METHODS

The study was conducted on the basis of the Department of Family Medicine and General Practice of Odessa National Medical University. By design, the study included 75 patients with asthma with varying degrees of severity, persistent course in the remission stage against the background of EBW or obesity. According to the randomization method, the patients were divided into 3 groups: the main group – 30 patients (the use of medication), the comparison group – 30 patients (training in the "Asthma School"), the 3rd group was controls of 15 patients (basic therapy), from the main group Stage 1 according to the same inclusion / exclusion criteria to complete the clinical trial, namely to improve the drug treatment against the background of basic treatment with the use of bacterial lysate and inosine pronabex drugs, together with training at the Asthma School.

The Asthma School training program included: a series of theoretical and practical classes where the patients received theoretical and practical skills in diet therapy, breathing and therapeutic exercises against the background of standard basic therapy.

The developed program of classes included 5 group and 9 individual classes. The following issues were considered during group classes: 1) The concept of asthma, anatomy and physiology of the respiratory system; 2) basic therapy of asthma; 3) the role of inhaled corticosteroids in the treatment of asthma; 4) exacerbation of asthma and its therapy. Night asthma; 5) non-medication methods of asthma treatment.

Individual classes included recommendations on: diet therapy, breathing exercises, therapeutic exercises.

Together with the Asthma School study, the patients in the main and comparison groups received self-monitoring diaries, which they filled in at home daily and brought to revisits to the doctor. The diary looked like tables where patients had to note the intake of medications, the implementation of breathing exercises, the frequency of taking rescue medications, waking up at night because of attacks of asthma, limitation of physical activity due to asthma. Each sheet also had the telephone number of the doctor who supervised the patient while studying at the Asthma School.

The control of the patient's disease and responsibility was performed according to the following criteria: understanding of the nature of the disease, methods of control and treatment of asthma, technique of using inhaler devices for drug delivery, adherence to the basic treatment, number of taking rescue drugs, namely short-acting β-2-agonists (SABA), decrease in BMI, controlled by bioempidansometry and weigh-scales –by OMRON BF 51 impedance meter and, most importantly, improvement in asthma control, by the Asthma Control Test (Quality Metri c Incorporated, 2002).

Statistical processing of the results was performed using parametric and non-parametric analysis methods. In the comparative analysis of independent groups, we used the Student's criterion for unpaired samples (subject to homoskedasticity and normal data distribution) and the Mann-Whitney test (for heteroscedastic data with a different type of distribution).

Sign correlation was studied by Spearman's (r) correlation analysis, and Pearson's criterion χ^2 was used to evaluate the relationship between qualitative and quantitative signs.

Patients' participation in this study was based on the written consent. There were used only medicines and licensed techniques registered in Ukraine. The study was conducted to preserve the confidentiality of personal information about patients.

RESULTS

According to the anthropometric study, 75 patients enrolled in the study were found to have EBW or obesity and the mean BMI was 31.67 ± 0.53 kg / m^2. The patients had varying degrees of asthma severity, 15 patients with a mild degree, 35 with a moderate degree, and 25 – with severe BA. Most of them were women, namely 40 and 35 men. A Spearman correlation coefficient of $\rho = 0.376$ was established, between the severity of the course and sex of the patients, the relationship between the studied signs was direct, and the closeness (constraint) force by the Cheddock scale was moderate.

The mean age of the patients was 43.93 ± 1.15 years. When conducting a correlation analysis between the age and severity of asthma it was found that the correlation coefficient according to Spearman was $\rho = 0.541$, the relationship between the studied signs – direct, closeness (constraint) force by the Cheddock scale was noticeable.

Respiratory exercises were performed by 14 (46.67%) patients in the main group and 16 (53.33%) patients in the comparison group, but only in the primary diagnosis of asthma, at the time of the study, no patient practiced breathing exercises.

Table I. Frequency of taking rescue medications per week in patients with bronchial asthma against the background of excess body weight or obesity in the course of application of the treatment-and-prophylactic complex

Group	Follow-up period	The number of taking SABA per week
Main group	Before	7±1.2
	After	2±0.5*
Comparison group	Before	7±1.5
	After	4±1.9
Control group	Before	6±1.5
	After	6±1.2

Note: *p before-after<0.001

Table II. Body mass index and body component indices in patients with bronchial asthma against the background of EBW or obesity

Index	Catamnesis stage	Main	Comparison
IMT kg/m^2	Before using TPC	32.00±0.85	31.88±0.87
	In 32 weeks after using TPC	29.7±0.74*	29.8±0.73
%fat	Before using TPC	38.68±1.40	39.20±1.30
	In 32 weeks after using TPC	36.51±1.26	36.59±1.28
Visceral fat	Before using TPC	12.33±0.76	11.93±0.83
	In 32 weeks after using TPC	11.39±0.57	11.41±0.71

Note: *p before-after<0.05

It was also found that 20 (66.67%) patients in the main group and 21 (70.00%) patients in the comparison group did not regularly use basic therapy and cancelled or changed the drugs without permission. However, it was found that the vast majority of patients, namely 42 (70.00%) of both groups, did not follow the correct technique of inhalation drug delivery.

At the beginning of the study, 21 (70.00%) patients in the main group and 15 (50%) patients in the comparison group indicated that they did not have sufficient knowledge and motivation to keep the prescribed treatment. However, after the use of the medication, 26 (86.67%) patients in the main group and 24 (80.00%) patients in the comparison group noted that they had improved knowledge of asthma, treatment and asthma control. At the same time, 90% of the patients in the main group and the comparison group (n = 54), after having motivational interviews, filling in the diary, achieving compliance with the doctor, acknowledged that greater responsibility for the control of the disease and adherence to administration rests on their shoulders.

In our country, there is a widespread practice of prescribing and using rescue drugs SABA as a monotherapy for BA, which by the principles of evidence-based medicine is absolutely irrational and has a negative impact on the course and control of the disease. The same trend was observed in our patients. After the Asthma School classes, the patients significantly reduced their use of SABA (Table I), which indicated a more conscious attitude toward basic therapy and improved compliance, which in turn indicated an increased sense of responsibility in this group of patients.

Another urgent issue that was solved at the individual class was teaching the patient how to use the inhalation device correctly. So, we have found that only 25 (33.33%) of 75 patients enrolled in the study were able to use inhalation drug delivery device properly. Also, most patients did not know that there were different means of delivery of inhalation drugs, so we were individually selected inhalation drugs according to the international recommendations and wishes of the patient and selected a separate individual class in which the patient together with the doctor studied the stages of inhaler use. After the training, 45 patients (76.66%) of the total patients of the main group and the comparison group began to perform respiratory movements with the inhalation device correctly.

During the follow-up period, positive dynamics of BMI reduction, percentage of the adipose tissue and visceral fat were observed in the patients who were trained in the Asthma School (comparison group) and in the patients treated with the developed TPC (Table II).

The table II shows that patients in the main group reduced body weight on an average by 4.5 kg, and BMI from 32.00 kg / m^2 changed to 29.7 kg / m^2 ($p < 0.05$), although they did not reach 25.0 kg / m^2 of the target level because the follow-up period was not long. The patients in the comparison group also had a positive result, namely a decrease in the body weight on an average by 2.5 kg during the follow-up period and reduced BMI from 31.32 kg / m^2 to 29.8 kg / m^2 ($p>0.05$), although it was not as reliable as in the main group. The patients in the control group had no changes in BMI and even a small number of patients, namely 3 patients (20%) increased their body weight by 1.9 ± 0.9 kg for 32 weeks of the follow-up.

We noted a tendency for a decrease in the body weight and visceral fat in the patients of the main group and the comparison group, but these changes were not statistically significant ($p > 0.05$).

Table III. The number of steps per day in the dynamics of following-up patients with bronchial asthma against the background of EBW or obesity

Groups	Before using TPC pressure/day	In 32 weeks after using TPC pressure/day
Main	1.800±0.17	7.600±0.22*
Comparison	1.930±0.16	5.900±0.31*
Control	1.870±0.26	2.100±0.52

Note: *p before-after<0.001

Table IV. Changes in the result of the AST-test according to the severity of the course in patients with bronchial asthma against the background of EBW or obesity in the course of application of the treatment-and-prophylactic complex

Group	Degree of severity	Before using TPC	In 20 weeks after using TPC
Mai group	Mild	16.54±0.49	20.16±0.75*
	Moderate	13.97±0.64	17.08±0.52*
	Severe	11.88±0.34	16.16±0.98*
Comparison group	Mild	16.61±0.53	17.98±1.01
	Moderate	14.01±0.97	15.45±0.34
	Severe	11.75±0.81	12.88±0.77
Control group	Mild	16.52±0.38	16.66±0.67
	Moderate	13.99±0.76	14.01±0.45
	Severe	12.05±0.63	11.96±0.50

Note: *p before-after<0.05

It is well known that controlled dosed physical activity (CDPA) has a positive effect on asthma. Our patients were aware of this but did not follow this postulate. While analysing physical activity it was found that none of the patient visited the sports sections or the gym, sometimes physical exercises were performed at home by 23.33% (n = 14) of the patients of the main group and the comparison group. According to the pedometer, at the beginning of our study, the majority of patients, namely 54 (72%), took no more than 2 thousand steps per day. At the group and individual classes, recommendations were given to increase CDPA, in the form of short-term and clear goals. The results of the follow-up are shown in the table III.

The table III shows the positive dynamics in increasing the physical activity of the patients in the main group and the comparison group, but it was noted that the patients in the comparison group took less steps per day than patients in the main group, which was associated with deterioration of health, so we chose the tactics maintaining the results obtained, rather than increasing them, for the period until the patients' well-being was stabilized, with further prolongation of CDPA.

It should be noted that a decrease in BMI and an increase in CDPA were due to a more honest attitude of the patients on their health and lifestyle, indicating an increased sense of responsibility in the patients included in our study.

To assess disease course control, we selected a simple and rapid test to evaluate the disease course over the last 4 weeks, namely the AST test. The patients filled in the printed questionnaires together with the physician on their first visit, 20 weeks after TPC and 32 weeks after TPC. The results in the Table 4 reflect the average AST test according to the severity of BA in the three study groups.

The table IV shows that only patients in the main group had significant positive changes in asthma control, the patients in the comparison group also had positive dynamics, but it did not show statistically significant differences. In the control group, they did not differ during the follow-up period and had approximately stable AST test results. In the questionnaire survey at the last visit, which was in 32 weeks after the end of TPC, it was found that about 20% of the patients in the main and comparison groups reported a slight regression in wellness and in asthma control, respectively.

The obtained positive dynamics according to the long-term results over 32 weeks of the follow-up proved the effectiveness of the proposed program in improving asthma control in patients in the main group RR = 0.38; RRR = 0.62, NNT = 2.12; the patients in the comparison group had the following indices – RR = 0.89; RRR = 0.11; NNT = 4.05. However, it should be noted that 90% of the patients noted an increase in responsibility for their health, course and asthma control, which is very important in the physician-patient relationship in today's world.

DISCUSSION

In our article, the topic was fully disclosed. The results of the research work are original, since in the literature of Ukraine this topic is not disclosed. It is necessary to introduce this technique into the practice of doctors, not only in the treatment of bronchial asthma, but also in the treatment of other chronic diseases. This article may be a prototype for research in other medical specialties.

CONCLUSIONS

1. The patient's low educational attainment regarding the course, control and treatment of the disease is one of the problems in achieving physician-patient compliance on the path to asthma control.
2. The use of rescue drugs is irrational among the asthmatic population in Ukraine, which can be solved by raising awareness and responsibility for the disease and methods of treatment of asthma.
3. Responsible attitude of the patient to nutrition, controlled dosed physical activity influences the decrease in the body weight ($p<0.05$) and improves the patient's health, which is one of the key points in the improvement of asthma control.
4. Self-completion of the patient's diaries disciplines the patient and makes his / her attitude to BA and disease control more responsible in 90% of cases, which is confirmed in our investigated improvements in the AST test results ($p<0.05$).
5. Studying at the Asthma School together with pharmacological correction has a positive effect on the course of asthma and its control (RR = 0.38; RRR = 0.62, NNT = 2.12).
6. The doctor should be interested in the fact that at the first and subsequent visits regarding the disease, in our case it is bronchial asthma, the patient will feel responsible for the disease. Therefore, when communicating with the patient, the physician should focus on this, provide the most complete answers to the patient's questions, provide educational literature and, if necessary, diaries of self-control.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

COGNITIVE DISORDERS IN PERSONS OF WORKING AGE WITH DYSCIRCULATORY ENCEPHALOPATHY

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ABSTRACT

The aim of the study was to study the neuropsychological features of cerebrovascular disorders in persons of working age at the outpatient clinic stage.

Materials and methods: 90 persons of working age were surveyed. Clinical neurological and clinical instrumental examination.

Results: The first group consisted of patients with dyscirculatory encephalopathy on the background of arterial hypertension – 60 people, the second with dyscirculatory encephalopathy on the background of cerebral atherosclerosis – 30. Assessment of cognitive function was examined using the MMSE Mental Status Scale, Anxiety, and Depression Scale using the DASS-21 scale.

Conclusions: According to the results of the study, significant memory impairment on the MMSE scale ($p < 0.003$) was found in patients with dyscirculatory encephalopathy without arterial hypertension. In patients with dyscirculatory encephalopathy on the background of hypertension, anxiety ($p < 0.001$) and depressive disorders ($p = 0.033$) were significantly more prevalent compared with patients with dyscirculatory encephalopathy without arterial hypertension. The increase in depressive disorders and anxiety in patients with dyscirculatory encephalopathy on the background of arterial hypertension decreases orientation and memory.

KEY WORDS: hypertensive dyscirculatory encephalopathy, hypertension, memory, anxiety, depressive disorders

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INTRODUCTION

Decreased cognitive function is one of the most common and socially significant disorders of the nervous system. A high level of cognitive and intellectual functions is a positive prognostic feature of life expectancy. Cerebrovascular pathology, neurodegenerative diseases and their combination are the main causes of cognitive deficit [1, 2, 3, 4]. Prevalence of cognitive disorders is associated with the current tendency to increase life expectancy and growth of the elderly people in the population, as well as to increase the occurrence of major risk factors for cognitive decline such as arterial hypertension, cerebral atherosclerosis and diabetes mellitus, which determines relevance of this problem for neurologists and doctors of other specialties [5].

Cardiovascular risk factor for cognitive impairment is metabolic syndrome, which includes: arterial hypertension, dyslipidemia, obesity, diabetes mellitus, impaired carbohydrate tolerance, underlying pathological mechanisms of cardiac and cerebrovascular disease. Recently, the prevalence of metabolic syndrome has been observed among able-bodied young and middle-aged people, which makes this problem extremely urgent [6, 7].

At present, arterial hypertension is considered as the most significant risk factor for the development of both acute and chronic disorders of the cerebral circulation, as well as disorders of memory and cognitive disorders. In many studies the relationship of persistent increase in blood pressure with the risk of progression of cognitive disorders has been proven [8]. Also, a large number of studies have shown that the predominance of dementia is a predominant increase in systolic pressure [7]. Chronic cerebral hypoperfusion due to increased vascular resistance in patients with hypertension may be a major factor in the development of dementia in hypertension. The brain is dependent on adequate oxygen and glucose delivery, and a decrease in cerebral blood flow impairs neuronal function, with possible subsequent brain damage [9].

The most common pathogenetic cause of cognitive impairment is the «small vessel disease» of the brain, which is more common in patients with hypertension and diabetes [10]. One of the substrates for the development of «small vessel disease» is atherosclerosis, which leads to the loss of smooth muscle cells tunica media, deposition of fibrogialin substances, narrowing the lumen and thickening of the vessel wall (lipogialinosis). In a more pronounced process,

Table I. Frequency of cognitive impairment of varying degrees of expression on the MMSE scale.

Index (in points)	Patients with hypertensive dyscirculatory encephalopathy, n = 60 (%).	Patients with dyscirculatory encephalopathy, n=30 (%).
Dementia of moderate severity on the MMSE scale (11-19)	-	-
Dementia of mild severity on the MMSE scale (20-23)	-	-
Cognitive impairment on the MMSE scale (24-27)	24(40)	14(46,7)
Normally, cognitive impairment is absent on the MMSE scale (28-30)	36(60)	16(53,3)

Table II. Results of the MMSE study.

	Patients with hypertensive dyscirculatory encephalopathy M(Q1-Q3)	Patients with dyscirculatory encephalopathy M(Q1-Q3)	P
Orientation	10 (9-10)	10 (10-10)	0,217
Memory	3 (3-3)	3 (2-3)	0,003
Counting operations	3 (3-3)	3 (3-3)	0,897
Overall cognitive performance	28 (26-28)	28 (27-28)	0,597

fibrinoid necrosis of the vascular wall leads to rupture of vessels and micro- or macro-hemorrhages, more often in the basal ganglia or thalamus [11].

Thus, the problem of chronic disorders of cerebral circulation and cognitive function in patients with hypertension is relevant in modern angioneurology. It should be noted that the number of patients with chronic disorders of the cerebral circulation, especially against the background of hypertension, is virtually none, which determines the relevance of the problem of further diagnosis and treatment.

THE AIM

The aim of the study is to study the neuropsychological features of cerebrovascular disorders in persons of working age at the outpatient clinic stage.

MATERIALS AND METHODS

Under our observation, there were 90 people aged 40 to 68 at the State Institution of Science «Research and Practical Centre of Preventive and Clinical Medicine» State Administration Department, Kyiv, Ukraine.

Patients were divided into two groups statistically comparable by major disease – dyscirculatory encephalopathy, sex and age. The first group consisted of patients with dyscirculatory encephalopathy and hypertension – 60 people, the second with DE on the background of CAC – 30. Among the examined patients in the first group were 17 men and 43 women. In the second group – 9 men and 21 women. The mean age of men in the first group was 51.54±0.76, in the second 51.83±2.24, women in the first group 54.63±0.42, in the second 56.88±0.72.

Clinical-neurological and clinical-instrumental examination was performed for all patients in order to establish the stage and form of vascular-brain pathology.

The study did not include patients with severe somatic pathology, clinically significant vascular events, and history of traumatic brain injury. Clinical and laboratory study included general blood test, biochemical blood test, lipidogram. Clinical and instrumental examination methods included electrocardiography, blood pressure measurements, and heart rate.

To assess patients' cognitive function, they used: a short MMSE mental status rating scale (determining time and place orientation, status of short-term, long-term memory, language, gnosis, praxis) [12]. According to the MMSE, 29–30 points were assessed as no cognitive impairment, 27–28 points were mild cognitive impairment, 24–26 were moderate cognitive impairment, 20–23 points were initial dementia, <20 were pronounced stages of dementia.

Anxiety and depression were examined using the Depression Anxiety Stress Scales (DASS-21) [13]. The survey found normal, mild, moderate, severe and very severe depression, anxiety, and stress.

RESULTS

Based on the history of complaints, anamnesis data, as a result of clinical and instrumental examination in 90 patients revealed a symptom complex that meets the criteria for the diagnosis of manifestations of cerebral circulation, among patients of the first group in 19 (31.7%) were diagnosed dyscirculatory encephalopathy, stage I and in 41 patient (68.3%) – stage II of dyscirculatory encephalopathy. In the second group in 10 patients (33.3%) were diagnosed stage I of dyscirculatory encephalopathy and in 20 patients (66.7%) – stage II. Arterial hypertension was observed in all patients in the first group. According to this classification 7 (11.7%) patients had stage I hypertension and 53 (88.3%) – arterial hypertension, stage II.

After conducting a neuropsychological test on the MMSE scale, 40% of patients with hypertensive dyscirculatory

Table III. The results of the study on the scale DASS-21.

	Patients with hypertensive dyscirculatory encephalopathy M (Q1-Q3)	Patients with dyscirculatory encephalopathy M(Q1-Q3)	P
Stress	11 (9-14)	11 (10-15)	0,372
Anxiety	9 (8-10)	7 (7-8)	0,001
Depression	9 (8-10)	8 (8-9)	0,033

Table IV. Characterization of the correlation between the cognitive function on the MMSE scale and the indicators of emotional-volitional function on the DASS-21 scale in patients with hypertensive dyscirculatory encephalopathy.

Indicators	Depression		Anxiety		Stress	
	r	p	r	p	r	p
Overall cognitive performance index	**-0,348**	**0,0064**	**-0,337**	**0,0084**	-0,073	0,5782
Orientation	**-0,327**	**0,0122**	**-0,335**	**0,001**	-0,17	0,2008
Memory	**-0,287**	**0,0261**	**-0,398**	**0,0016**	-0,154	0,24

r - Spearman rank correlation index.

Table V. Characteristic of the correlation between the cognitive function on the MMSE scale and the emotional-volitional function on the DASS-21 scale in patients with dyscirculatory encephalopathy.

Indicators	Depression		Anxiety		Stress	
	r	p	r	p	r	p
Overall cognitive performance index	-0,324	0,1319	-0,303	0,16	-0,187	0,3935
Orientation	0,042	0,8505	0,025	0,9093	0,154	0,484
Memory	**-0,447**	**0,0326**	-0,367	0,0849	-0,394	0,0629

r - Spearman rank correlation index.

encephalopathy and 47.8% with dyscirculatory encephalopathy were found to have cognitive impairment (Table I). A significant difference, on the MMSE scale, p <0.003 by Wilcoxon's W test in the two surveyed groups was observed in the memory study (Table II). In the study of memory, the incidence rate of X2 = 0.02 significantly prevailed in patients of the first group – 15% of memory was assessed at 2 points, at 85% – 3 points. At the same time, 48% of patients in the second group had 2 points, 52% had 3 points.

The significant decrease in memory level was mainly due to the impairment of self-reproduction of information that was manifested in operational activities and training, while the memory of life events remained largely preserved. Such modal-nonspecific mnestic disorders with primary disorders of short-term memory in combination with fatigue and emotional instability indicate the defeat of the middle nonspecific structures of the brain at the level of the lower parts of the brain stem (I-th functional block of the brain according to Luriya AR [14].

It can be assumed that the irregularities of the calculus operations recorded initially are not related to organic changes of the frontal lobes of the brain (primary and secondary «frontal acalculia»), but depend on the lesions of the basal and medio-basal divisions of the frontal lobes of the brain (limbico-hippocampal system), which is known to lead to significant changes in the emotional sphere of the patient's behavior [15]. Defects of this type of intellectual activity – counting operations, in our category of patients arise on the basis of impulsivity and manifest themselves in violation of the orienting basis of action by reducing attention. Errors in them occur due to the acceleration of the flow of nervous and mental processes due to defects in the inhibitory processes [16] and/or cholinergic dysregulation [17].

After testing on the DASS-21 scale, it was found that mild depression was observed in 35% of patients with hypertensive dyscirculatory encephalopathy, moderate anxiety in 50%, severe anxiety in 16.7%; in patients with dyscirculatory encephalopathy mild depression – 8.7%, mild anxiety – 39.1%. Significant difference on the DASS-21 scale of anxiety p <0.001 and depression p = 0.033 between the indicators of the two groups according to Wilcoxon's W criterion was shown in Table III.

Such changes in the level of anxiety should be associated, probably, with the recovery after treatment of neurohumoral balance: GABA-ergic inhibitory effects, serotonin, dopamine-peptidergic activation systems [18].

In most cases, the law of distribution of values was different from the normal one, so Spearman's rank correlation index was calculated for correlation analysis.

A negative correlation between the mean severity of depression and anxiety and overall cognitive performance (r = –0.348, p = 0.006), (r = –0.337, p = 0.008) was found in patients with hypertensive dyscirculatory encephalopathy. As depression and anxiety increase, the overall cognitive performance index decreases. The negative correlation was

found in the mean severity of depression and anxiety and orientation (r = –0.327, p = 0.01), (r = –0.335, p = 0.001), respectively, with increasing depression and anxiety the orientation value decreases. Also, a negative correlation was found between the mean severity of depression and anxiety and memory (r = –0.287, p = 0.02), (r = –0.398, p = 0.001), respectively, with increasing index depression and anxiety the value of memory decreases (Table IV).

Patients with dyscirculatory encephalopathy on the background of cerebral atherosclerosis revealed a negative correlation between the mean severity between depression and memory (r = –0,447, p = 0.03), the value of depression memory decreases (Table V).

CONCLUSIONS

1. Significant memory impairment on the MMSE scale (p <0.003) was found in patients with dyscirculatory encephalopathy without arterial hypertension compared with patients with dyscirculatory encephalopathy on the background of hypertension.
2. In the study of emotional and volitional disorders in persons of working age, it was proved that in patients with dyscirculatory encephalopathy on the background of arterial hypertension, anxiety (p <0.001) and depressive disorders (p = 0.033) significantly outweighed compared with patients without arterial hypertension.
3. It is proved that the increase of the index of depressive disorders and anxiety in patients with dyscirculatory encephalopathy against the background of arterial hypertension decreases the index of orientation and memory.

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ORIGINAL ARTICLE
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SOCIOLOGICAL STUDY RESULTS OF SELF-ASSESSMENT POSSIBILITIES FOR SELF-REALIZATION AMONG DOCTORS OF GENERAL PRACTICE – FAMILY MEDICINE IN UKRAINE

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ABSTRACT

The aim: To study self-assessment opportunities for primary care doctors' self-realization and their satisfaction with work during health care transformations in Ukraine.

Materials and methods: To achieve this purpose, we compiled a questionnaire according to which an anonymous survey was conducted among 247 family doctors from six regions of Ukraine (Cherkasy, Zakarpattia, Odessa, Kirovohrad, Sumy, Kiev regions). In the study, we used the following research methods: sociological and medical-statistical. The method of mathematical statistics by applying Microsoft Excel 2013 was used to analyze research outcomes.

Results: The obtained data on self-assessment opportunities for primary care doctors' self-realization show that most of the respondents are not satisfied with their work. At the same time, the older general practitioners – family doctors are, the less important self-realization becomes for them, due to a disturbing fact of a highly probable lack of job, which is the first priority problem they may face. The willingness to work abroad is reported more than twice as high among family doctors aged up to 47 years. According to family doctors, irrespective of their age, the priority problems to be solved were reported as follows: poor financings; insufficient material and technical resources of out-patient clinics of the general medical practice; the necessity of returning national clinical protocols; the incompetence of health management; not clearly established interconnection between different levels of health care system.

Conclusions: Family doctors in Ukraine have mostly unsatisfactory conditions for their self-realization. This fact has a significant influence on their willingness to change a job, especially among doctors of young age.

KEY WORDS: family doctor, self-realization, sociological research

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INTRODUCTION

The health of the population is determined on a state level as one of the highest values. Health care sector in Ukraine, mainly state-funded, is designed to provide available, high-quality, effective, timely, free medical care to each citizen. The affordability of health care, which has always been a national policy priority, is first of all realized through rendering primary medical care. The experience of the developed countries in the world demonstrates the proven economical and medical efficiency of primary medical care development based on the family medicine principles [1-5].

The international Health 2020 Strategy, which Ukraine also joined, determined effective vectors for noninfectious diseases prevention (causing 86.0% of death in the world) and factors of healthy lifestyle formation, health preservation and promotion for the population of all ages taking into account their regional features.

The family medicine is acknowledged to be a health reform priority in Ukraine, however, the development and strengthening of primary care staffing remains an unresolved strategic problem at the state level.

THE AIM

To study self-assessment opportunities for primary care doctors' self-realization and their satisfaction with work during health care transformations in Ukraine. To find out the willingness of primary care doctors to participate in reforms, their workplace satisfaction, the availability of opportunities for self-realization and (or) their willingness to change a job or even the country (to go abroad).

MATERIALS AND METHODS

To achieve the purpose of our study, we compiled a questionnaire which included six groups of questions regarding gender and age characteristics of respondents; places of their medical practice (city / village); opportunities for professional self-realization, the determination of their job importance among other priorities; their workplace satisfaction including the organization of work at the family medicine out-patient clinic; their willingness to change a job , or even the country to practice medicine (to go abroad); proposals for further primary care reforming in Ukraine.

The anonymous survey was conducted among 247 family doctors from 6 regions of Ukraine (Cherkasy, Zakarpattia, Odessa, Kirovohrad, Sumy, Kiev regions). The average age of respondents was 47,0±0,9 years. In the study there were used sociological and medical-statistical research methods.

The method of mathematical statistics by applying Microsoft Excel 2013 was used to analyze research outcomes.

RESULTS

The acknowledgement of primary health care based on the general medical practice principles to be a Ukrainian health care priority demands scientific maintenance for its introducing into practice. The obtained research data allowed us to state problems which have potentially negative impact on the organization of rendering primary medical care to the population. Among them, the most important is a problem of insufficient primary care staffing without clear state strategy for its planning, which can be explained by a low motivation of primary care doctors to work effectively, their unwillingness of self-realization due to the government-related uncertainty in organizing their work.

Among the interviewed family doctors (247 respondents), who are members of the public organization "The Ukrainian Association of Family Doctors" from six regions of Ukraine (Cherkasy, Zakarpattia, Odessa, Kirovohrad, Sumy, Kiev regions), family doctors from Sumy (33,2 ± 3,0%) and Zakarpattia (21,1 ± 2,6%) regions occurred to be the most active. An average age of respondents was 47,0 ± 0,9 years, including the youngest age of 23 and the oldest age of 72. The majority of the interviewed were family doctors having medical practice in the cities (59,8 ± 3,1%).

The obtained sociological data on family doctors' self-assessment of their possibilities for self-realization show that majority of respondents reported the work availability as more important factor (86,0 ± 2,2%, at the maximum of 100,0% of respondents from Odessa, Kirovohrad, Kiev regions and the minimum of 67,3 ± 6,5% in Zakarpattia region) if compared to the possibility of professional self-realization (64,1 ± 3,1%, rating from 93,8 ± 4,3% among respondents from Kirovohrad region up to 9,1% ± 8,7% of them from Kiev region).

According to the age distribution, the respondents of over 47 age group reported the lowest willingness for self-realization if compared to those who were younger (61,6 ± 4,4% against 80,3 ± 3,6% among doctors aged up to 47 years).

The analysis of respondents' answers to questions about their work satisfaction showed no significant difference in age groups, every second family doctor is not satisfied with their work (48,4 ± 4,5% in the age group of up to 47 and 49,6 ± 4,5% in the age group of over 47).

According to the regional distribution, it should be noted that a higher number of family doctors being satisfied with their work is from Kirovohrad (75,0 ± 7,7%) and Cherkasy (69,2 ± 7,4%) regions and the lowest number of them is from Sumy (17,1 ± 4,2%) and Kiev (18,2 ± 11,6%) regions.

Under the existing circumstances in Ukraine, practically every third primary care doctor in Cherkasy (28,2 ± 7,2%), Odessa (25,8 ± 7,9%), Kirovohrad (28,1 ± 7,9%), Kiev (36,4 ± 14,5%), Sumy (41,5 ± 5,4%) and fewer respondents from Zakarpattia (19,2 ± 5,5%) regions are willing to change even the country of medical practice (to work abroad). The willingness to change the workplace and work abroad is twice higher among family doctors aged up to 47 (41,0 ± 4,5% against 20,8 ± 3,6% in other age group). At the same time, 79.2 3.6% of family doctors from the age group older than 47 years have no desire to work abroad.

Among the available medical care problems and their solutions during the difficult period of health care transformations in Ukraine, the highest number of respondents, irrespective of their age, reported as follows: uncertain and dramatic changes of organizational, economic and social conditions for citizens of Ukraine and the persons living on its territory, the insufficient primary medical care; poor financial support, in particular financial rationality and reasonable funds distribution; insufficient material and technical resources of out-patient clinics of the general medical practice; the necessity of returning national clinical protocols; incompetence of health care management; a lack of constructive compliance between health care levels; the insufficient legal protection of primary health care workers, and so forth.

DISCUSSION

The aim of studying self-assessment opportunities for primary care doctors' self-realization and their satisfaction with work during health care transformations in Ukraine was achieved. For the first time, the above mentioned survey among doctors of general practice – family medicine was conducted in Ukraine.

The revealed problems are stated to be highly relevant. The primary medical care doctors should be provided with good working conditions and the proper life level support to be able to render efficient primary medical care for the population as in 80.0% of requests. The primary care human resources component has been found out to be one of the main weak points of the health care system in Ukraine.

At the stage of modern health care sector reforming which began with the primary medical care level it is important to publish the obtained results, which will foster the fastest problem solutions. The policy of preserving young primary health care workforce and creating dignified working condition for it should be prioritized in Ukraine. The specificity of medical work demands continuing professional development and self-education which is significant for doctors to render efficient health care. The highly responsible profession of a doctor who provides efficient medical care to a patient is the key factor to be taken for granted in the health care comprehensive plans and investments to support proper working conditions for family doctors.

CONCLUSIONS

Thus, the development of primary health care remains the priority in Ukraine in spite of the fact that during the period of reforming its vectors have been changed to a certain extend. Planning the primary health care workforce and the quality of health workers' services should be supported by valid data and knowledge, taking into account the best

international and national practices and the Global Strategy on Human Resources for Health.

Modern conditions demand further work on creating legislative standards for functioning and further development of primary health care in Ukraine, which includes the government strategy for strengthening primary care human resources to avoid risks of loss of the young qualified health professionals.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

COMPARISON OF THE EFFECT OF DAPAGLIFLOZIN ON CONTRAST TO STANDARD THERAPY OF THE PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CONCOMITANT OBESITY, THEIR EFFECT ON LABORATORY AND ANTHROPOMETRIC PARAMETERS

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ABSTRACT

The aim: Evaluate clinical and laboratory parameters of the patients with type 2 diabetes mellitus and concomitant obesity after a course of dapagliflozin treatment and compare with a standard treatment regimen.

Materials and methods: Conducted a comprehensive clinical laboratory examination and measurement of the anthropometric parameters of the patients with type 2 diabetes mellitus and concomitant obesity, with subsequent statistical calculations.

Results: The data obtained at different stages of the study revealed a statistically significant effect of glucose treatment and glycosylated hemoglobin (HbA1c). Since the 6th month of dapagliflozin treatment, we have shown a tendency to lose weight compared to baseline in this group of patients and controls.

Conclusions: Type 2 diabetes mellitus and obesity significantly increase the risk of developing a number of complications. Complex control and effects on clinical laboratory and anthropometric parameters can statistically significantly influence the development of the complications, and in this context, dapaglifloflozin showed statistically better results than standard metformin monotherapy.

KEY WORDS: type 2 diabetes mellitus, obesity, treatment, diagnostics

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease characterized by insufficient amount of pancreatic insulin production, or the production of insulin is not used effectively [1]. According to the World Health Organization (WHO), as of 2016, about 8.5% (422 million) of the world's population suffer from T2DM worldwide [2]. It should be noted that with adequate health monitoring, about the 10–25% of T2DM can be prevented, including 2.34 million cases of depressive disorders [3].

In itself, T2DM is an extremely complex medical and social problem of today, since this disease contributes to a number of serious complications: limb amputation, loss of vision, cardiovascular disease, renal failure, damage to the nervous system, etc. [4].

The main diagnostic criteria for T2DM (according to the American Diabetes Association (ADA) 2020) [5]:

- 2-h fasting plasma glucose ≥200 mg/dL (11.1 mmol/L) during oral glucose tolerance test. (Using a glucose load containing the equivalent of 75 gram anhydrous glucose dissolved in water. In the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples) or
- Fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours or
- HbA1C ≥6.5% (48 mmol/mol) or
- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

There are a number of recommendations for the treatment of T2DM by The European Foundation for the Study of Diabetes (EFSD), ADA [6], International Diabetes Federation (IDF) [7], but despite some differences, they all agree on because the treatment should be complex and include: diet therapy, rational step-by-step pharmacotherapy (starting with metformin and depending on the level of glycosylated hemoglobin (HbA1c) after treatment – transition to combinations with the following drug groups), dosed physical activity and altered physical activity .

Dapagliflozin is a selective and inverse inhibitor of type 2 glucose cotransporter (SGLT2) [8]. Dapagliflozin lowers glucose levels both after ingestion and on an empty stomach by reducing renal glucose absorption and as a consequence of its excretion in the urine [8]. However, this medicine does not interfere with the normal production of endogenous glucose in response to hypoglycemia and acts independently of insulin secretion and action. Urinary glucose excretion induced by dapagliflozin is associated with calorie loss and weight loss [9]. Suppression of the co-transport of glucose and sodium by dapagliflozin may be accompanied by minor urine output and temporary excretion of sodium by urine [8].

A number of the clinical trials (CDs) have been conducted, the analysis of the obtained data of which testify to the safety of this medicine. One of the CDs is the cardiovascular events (DECLARE) assessment with dapagliflozin at a dosage of 10 mg compared with placebo with the participation of 17,160 patients with T2DM with / without established cardiovascular disease to evaluate the effects on the cardiovascular events [10]. CVD-REAL 3 Multinational Cohort Study Data Analysis, which included the information on more than 65,000 patients in Israel, Italy, Japan, Taiwan and the United Kingdom, the primary purpose of which was to compare SGLT2 drugs and other antipyretics, and included measurements and estimation of glomerular filtration rate (eGFR) before and after (within 180 days) [11]. The study provided global, real-world evidence for the evaluation of the efficacy and safety of SGLT2 drugs, in particular closely related to a lower risk of major renal events [11].

According to the 2016 WHO data, approximately 1.9 billion people of the age 18 and over are overweight, 600 million of whom are obese [12]. At present, 35-36% of men, 41% of women and 15-16% of children have obesity or overweight in Ukraine, while the tendency to increase of these indicators remains [13].

It should be noted that the consequences of obesity may be: cardiovascular diseases (hypertension, heart failure, atherosclerosis), neurological (stroke, polyneuropathy), musculoskeletal disorders (arthritis, psychiatric syndrome, pain syndrome) , eating disorders), gastrointestinal (gastroesophageal reflux disease, liver cirrhosis), endocrinological (T2DM, infertility), oncological diseases, etc. [14].

The main diagnostic criteria for obesity are measurements of body height and weight, followed by the BMI calculations. If BMI is≥30 kg/m^2, it can be regarded as obesity and therefore classified according to the degree of obesity [15]. Additionally measure the waist circumference (WC) and hip circumference (HC), followed by the calculation of their ratio (WC / HC). According to the WHO, the WC for men is 94 cm and for women is 80 cm, indicating that they are at increased risk of serious illness, while 102 cm and 88 cm respectively are regarded as extremely high risk [16]. The coefficient waist-to-hip ratio (WHR) obtained by the dividing WC (cm) by HC (cm) indicates health risks, but they increase dramatically if this ratio for men is above 0.95 for women – 0.85 [17]. Additionally, it is recommended to determine the thickness of the fatty fold. It is possible to instrumentally determine the extent of the distribution of adipose tissue in the human body by ultrasound, magnetic resonance imaging, computed tomography or bioimpandance, but these methods are expensive and do not require routine examination in each patient. For this reason, the search for new anthropometric indicators and obesity-related indicators is being continued, which will be able to better assess the degree of obesity and, accordingly, the distribution of adipose tissue [18, 19].

THE AIM

To evaluate the clinical and the laboratory parameters of the patients with T2DM and concomitant obesity after treatment with the dapagliflozin and compare with the standard.

MATERIALS AND METHODS

Selection of the patients with T2DM and a combination of obesity was performed on the basis of the hospital of the therapeutic department of the Municipal non-profit enterprise of the Uzhhorod Regional Clinical Hospital of the Uzhhorod Regional Council of Transcarpathian region and at outpatient treatment department of the therapy and the family medicine of the Faculty of Postgraduate And Pre-University Education of the State Higher Educational Establishment «Uzhhorod National University».

During November 2016 and January 2020, 137 patients were diagnosed with T2DM and concomitant obesity. The diagnosis criteria for T2DM were established on the basis of the ADA recommendations, but instead obesity was performed in accordance with the BMI≥30 kg/m^2 with subsequent distribution into groups by degree of the obesity (I, II and III degrees respectively).

All the patients underwent the comprehensive treatment, which included dietary therapy, dosed physical activities (at least 30 minutes per day) and pharmacotherapy with metformin 850 mg x 2 times daily in the I group (n = 71), instead the patients of the group II (n = 66) in addition to the complex treatment additionally received dapagliflozin 10 mg x once a day, and 18-month follow-up with 3 and 6-month follow-up, on the 12th, the 18th of the month.

The statistical processing of the research results was performed using the program software International Business Machines Corporation Statistical Package for the Social Sciences Statistics. The statistical analysis of the materials, the summary and also the summary of the conclusions were made by the method of the variation statistics, taking into account the average values (mod, median, arithmetic mean) and the average error (M±m), with the estimation of the reliability of the values by the Student's t-criterion, as well as with the determination of the correlation coefficient using the Pearson's paired method to identify the relationships between the obtained indicators. For the minimum threshold of probability, the values $p<0.05$ were taken.

The whole set of the surveys were in accordance with the Articles 3, 44 of the Fundamentals of the Legislation of Ukraine on Healthcare, the Articles 7, 8 of the Law of Ukraine "On Medicines", the Law of Ukraine "On Protection of Personal Data", taking into account the requirements of the European Parliament and Council Directives 2001 / 20 / EU of April 4, 2001, 2001/83 / EC of November 6,2001,the Decisions of the European Parliament and of the Council 1901/2006 of December 12,2006 and 1902/2006 of December 20,2006, ICH GCP, International Ethical Principles for Biomedical human-related research and physician code of conduct, and order in the Ministry of Health of Ukraine No. 690 of September 23, 2009, as well as the order of the Ministry of Health of Ukraine No. 1118 of December 21, 2012, unified clinical protocol of the primary and the secondary (specialized) medical care of T2DM.

RESULTS

Those included in this study were ≥40 years old. The mean age of the patients in the 1st group was 52.1 ± 1.1 years,

Table I. Anthropometrical parameters in group I and II.

	Group I (n=71)			Group II (n=66)		
	Total (n=71)	**Men's (n=25)**	**Females (n=46)**	**Total (n=66)**	**Men's (n=27)**	**Females (n=39)**
BMI ^{B} (kg/m^2)	32,64±0,19	32,54±0,27	32,70±0,25	32,74±0,22	32,44±0,37	32,94±0,28
WC ^{B} (cm)	109,97±1,44	110,96±1,15	109,43±2,14	109,06±1,38	110,62±1,17	107,97±2,19
HC ^{B} (cm)	104,0±0,98	99,88±0,96	106,3±1,31	103,7±1,02	99,29±0,81	106,8±1,45
WHR ^{B}	1,06±0,01	1,11±0,01	1,04±0,02	1,05±0,01	1,11±0,01	1,06±0,02
BMI ^{M3} (kg/m^2)	31,64±0,19	31,54±0,27	31,70±0,25	31,74±0,22	31,44±0,37	31,94±0,28
WC ^{M3} (cm)	107,5±1,43	108,5±1,22	106,9±2,12	106,7±1,39	108,4±1,19	105,5±2,19
HC ^{M3} (cm)	101,50±0,99	97,36±1,02	103,76±1,33	101,40±1,05	96,703±0,84	104,66±1,48
WHR ^{M3}	1,06±0,01	1,11±0,01	1,03±0,02	1,06±0,01	1,12±0,01	1,04±0,02
BMI ^{M6} (kg/m^2)	30,65±0,19	30,54±0,27	30,71±0,25	30,74±0,22	30,45±0,37	30,95±0,28
WC ^{M6} (cm)	104,2±1,44	105,4±1,24	103,6±2,12	103,6±1,40	105,3±1,35	102,3±2,18
HC ^{M6} (cm)	99,52±0,99	95,52±1,01	101,6±1,33	99,5±1,04	94,96±0,87	102,6±1,46
WHR ^{M6}	1,05±0,01	1,10±0,01	1,02±0,02	1,05±0,01	1,11±0,01	1,03±0,02
BMI ^{M12} (kg/m^2)	30,47±0,16	30,45±0,22	30,51±0,25	29,79±0,22*	29,63±0,32*^{1}	30,15±0,24*^{2}
WC ^{M12} (cm)	103,5±1,32	103,1±1,27	102,4±2,12	101,2±1,40	103,3±1,33*^{1}	100,3±2,14*^{2}
HC ^{M12} (cm)	97,35±1,32	94,71±1,01	99,3±1,42	97,1±1,05	93,96±0,88	100,1±1,39*^{2}
WHR ^{M12}	1,03±0,02	1,09±0,02	1,01±0,01	1,01±0,02*	1,05±0,01*^{1}	1,01±0,02
BMI ^{M18} (kg/m^2)	30,31±0,23	30,41±0,12	30,47±0,29	29,72±0,29*	29,59±0,37*^{1}	29,87±0,25*^{2}
WC ^{M18} (cm)	101,4±1,28	101,4±1,31	101,2±2,09	101,1±1,32*	102,7±1,33*^{1}	97,2±1,99 *^{2}
HC ^{M18} (cm)	95,78±1,21	93,68±1,2	97,3±1,33	94,57±1,03	91,63±0,88	97,6±1,25
WHR ^{M18}	1,02±0,01	1,07±0,01	1,00±0,01	1,00±0,03*	1,03±0,02*^{1}	1,00±0,05*^{2}

Note: B - patient data at baseline; M - 3, 6, 12, 18 - patient data for 3, 6, 12, 18 months of observation of the respective patient groups; * - statistically significant difference when comparing the indicators between the respective groups I and II ($p < 0.05$); where * 1 - between men and * 2 between women; BMI - Body Mass Index; WC - Waist circumference; HC - The hip circumference; WHR - waist-to-hip ratio.

compared with 54.5 ± 1.2 years of the patients in the 2nd group ($p > 0.05$). The ratio of men and women in the group I is 25 men and 46 women versus 27 men and 39 women in the group II. The mean duration of T2DM in group I was 15.3 ± 2.1 years, as opposed to 16.7 ± 1.9 years.

According to the data obtained as a result of the measuring anthropometric parameters of the patients of the 1st and the 2nd group at the beginning of the study, no statistically significant difference was found between them ($p > 0.05$). The BMI at the beginning of the study in the group I was 32.64 ± 0.19 kg/m2, respectively, 32.74 ± 0.22 kg/m^2 in the group II. The WC index in group I at the beginning of the study was 109.97 ± 1.44 cm, respectively 109.06 ± 1.38 cm in the group II. WHR in the group I was 1.06 ± 0.01 and 1.05 ± 0.01, respectively, in the group II.

It is noteworthy that, starting from 12 months after the completing the course of the complex treatment and at the 18th month, the visit of the completion of the treatment and observation, between the anthropometric parameters (AP) of the patients of the 1st and the 2nd group, there was a statistically significant difference, the same the dynamics were also observed when comparing the sex between the two groups ($p < 0.05$).

Analyzing the biochemical parameters of the blood, namely hydrocarbon metabolism, there is a tendency to decrease fasting plasma glucose (FPG) and HbA1C, but the target HbA1C levels were reached only at the 18th month of the comprehensive treatment. At the same time, statistically significant results were recorded when the comparing results of the I and the II groups ($p < 0.05$), as well as when comparing by sex ($p < 0.05$), despite the fact that at the beginning of the study there was no statistically significant difference ($p > 0.05$).

The other biochemical parameters obtained at different stages of the study did not reveal the statistically significant changes in the indicators of the groups I and II ($p > 0.05$).

A rather interesting finding at the beginning of the study was that thyroid hormone levels in the group I revealed 22% of the patients with the subclinical hypothyroidism (SCH), while in the group II it was detected in 27% of the patients.

DISCUSSION

In spite of the data obtained by us, which indicate the safety of use of the dapagliflozin, the literature describes cases and incidence of the adverse events with the cardiovascular events and the bacterial urinary tract infections after the use of dapagliflozin of the patients with T2DM. Further studies will focus on the study and comparison of

Table II. FPG and HbA1C levels

	Group I (n=71)			Group II (n=66)		
	Total (n=71)	Men's (n=25)	Females (n=46)	Total (n=66)	Men's (n=27)	Females (n=39)
FPG ^{B}	8,94±0,14	8,86±3,71	8,99±3,74	8,90±0,13	8,77±3,71	8,99±3,7
HbA1C ^{B}	8,05±0,05	8,16±1,71	7,99±1,81	8,12±0,06	8,07±1,77	8,15±1,86
FPG ^{M3}	8,77±0,14	8,69±3,71	8,82±3,74	8,73±0,13	8,60±3,71	8,82±3,7
HbA1C ^{M3}	7,94±0,05	8,05±1,71	7,88±1,81	8,01±0,06	7,96±1,77	8,04±1,86
FPG ^{M6}	8,36±0,14	8,28±3,71	8,41±3,74	8,12±0,13	8,32±3,71	8,21±3,7
HbA1C ^{M6}	7,83±0,05	7,94±1,71	7,77±1,81	7,90±0,06	7,85±1,77	7,93±1,86
FPG ^{M12}	7,45±0,14	7,37±3,71	7,50±3,74	7,39±0,13 *	7,26±3,71 *^{1}	7,48±3,7 *^{2}
HbA1C ^{M12}	7,65±0,05	7,76±1,71	7,59±1,81	7,70±0,06 *	7,65±1,77 *^{1}	7,73±1,86 *^{2}
FPG ^{M18}	7,24±0,14	7,16±3,71	7,29±3,74	7,06±0,13 *	7,93±3,71 *^{1}	7,15±3,7 *^{2}
HbA1C ^{M18}	7,47±0,05	7,58±1,71	7,41±1,81	6,39±0,06 *	6,40±1,77 *^{1}	6,38±1,86 *^{2}

Note: B - patient data at baseline; M - 3, 6, 12, 18 - patient data for 3, 6, 12, 18 months of observation of the respective patient groups; * - statistically significant difference when comparing the indicators between the respective groups I and II ($p<0.05$); where * 1 - between men and * 2 between women; Fasting plasma glucose - FPG.

FPG, HbA1C and AP levels with trace element and thyroid hormone levels, as well as the pharmacokinetic characteristics of this medicine. Additionally, a number of cases have been described in the literature when it is possible to use SGLT2 medicine as starting monotherapy, but these data are not yet sufficient for the routine clinical practice for the appropriate recommendations.

CONCLUSIONS

The incidence of these complications of the cardiovascular events and the renal complications can be reduced by providing patients with the adequate comprehensive treatment and controlling AP and hydrocarbon exchange, which may be indicative of the risk of their occurrence. The dapagliflozin study demonstrated the safety of its use and a slightly better effect on FPG, HbA1C, and AP compared to the standard metformin monotherapy. It is noteworthy that, starting from 12 months after the completing the course of the complex treatment and at the 18th month, the visit of the completion of the treatment and observation, between the anthropometric parameters (AP) and the hydrocarbon exchange (levels of FPG, HbA1C) of the patients of the 1st and the 2nd group, there was a statistically significant difference, the same the dynamics were also observed when comparing the sex between the two groups ($p<0.05$). A rather interesting finding during the study was the detection of SCH of the patients with T2DM and the concomitant obesity.

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COMORBIDITY IN PATIENTS WITH CHRONIC CORONARY SYNDROMES: PREVALENCE AND ASSESSMENT

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ABSTRACT

The aim: To carry out an evaluation of comorbidities in CCS patients having undergone PCI for stable coronary artery diseases (planned) or PCI in myocardial infarction (urgent) with the aim of justifying the best management strategies.

Materials and methods: We carried out an analysis of comorbid pathology spreading and the evaluation of multiple comorbidities using of Combined Age Charlson Comorbidity Index (CA-CCI) – in 138 patients below 75 yrs having undergone urgent and planned PCI. These patients were divided into two groups; the Ist one included 60 patients with urgent PCI (angioplasty with stent), the 2nd group – 78 patients with planned PCI.

Results: We found a high prevalence of comorbidity in CCS patients: the mean number of diseases in patients of the 1st and 2nd groups were 8.2±0.3 and 9.3±0.4, and the CA-CCI values – 7.3±0.3 and 7.5±0.2, respectively (p>0.05). Ten and more diseases were found in (28.3±5.8%) patients from the 1st group and in (43.6±5.6%) patients from the 2nd group (p=0.06). The most frequent comorbidities were arterial hypertension, cerebrovascular diseases, peripheral arterial disease, type 2 diabetes mellitus, and impaired glucose metabolism.

Conclusions: Comprehensive assessment and management of patients with CCS who undergo PCI remains a current problem in modern medicine, given the high prevalence of comorbid pathology.

KEY WORDS: chronic coronary syndromes, percutaneous coronary intervention, comorbidity, Charlson Comorbidity Index

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INTRODUCTION

A prominent feature of modern medicine is its continuous process of improvement concerning current diagnostics and treatment approaches. According to the recently published "2019 ESC Guidelines for the Diagnosis and Management of Chronic Coronary Syndrome" [1] the term "stable coronary artery disease" was substituted by "chronic coronary syndrome" (CCS). The authors point out "the dynamic nature of the coronary artery disease (CAD) process results in various clinical presentations which can be conveniently categorized as either acute coronary syndromes or chronic coronary syndromes" [1]. According to the indications of these Guidelines, comorbidity assessment belongs to the most important requirements for the clinical evaluation of patient's health status. It should be taken into consideration that presence of several diseases needing medical interventions leads to the worsening of patient's condition, increased disability and functional decline, and, as a result, to worsened patient's quality of life and life expectancy [2, 3]. Scientific researches having been carried out in different countries suggest the increasing amount of comorbidities in CAD patients [4, 5]. The results of longitudinal studies show a positive correlations between comorbidity evaluated according to the Charlson Comorbidity Index (CCI) and the life span. Among CAD patients, the most unfavorable prognosis is found for patients with type 2 diabetes mellitus, kidneys failure, chronic obstructive lung disease, and peripheral arterial disease [6]. Nowadays in clinical practice we can see an increasing number of CAD patients having undergone myocardial revascularization. There are here a lot of problems which are to be solved; consequently, the assessment of comorbid pathology spreading is an important one. In this study we carry out a combined evaluation of comorbidity in CCS patients after PCI for stable coronary artery diseases or PCI in myocardial infarction in order to justify the need for their management optimization.

THE AIM

To carry out an evaluation of comorbidities in CCS patients having undergone PCI for stable coronary artery diseases (planned) or PCI in myocardial infarction (urgent) with the aim of justifying the best management strategies.

MATERIALS AND METHODS

We performed a retrospective analysis of 138 electronic medical records of CCS patients under age 75 years having obtained their medical care at the State Institution of Sciences "Research and Practical Center of Preventive and Clinical Medicine" State Administrative Department. All

patients underwent myocardial revascularization via PCI for CAD (planned PCI) or PCI in myocardial infarction (angioplasty with stent, urgent PCI). The patients were divided into two groups; the 1st group includes 60 persons (45 males and 15females) having undergone urgent PCI (patients were included to the study in a year or later following revascularization); the 2nd group includes 78 persons (59 males and 19 females) after planned PCI. All the patients had CCS (according to the "2019 ESC Guidelines for the Diagnosis and Management of Chronic Coronary Syndrome") [1]. Patient`s age ranged from 34 to 75 years, the mean age of patients in the 1st and 2nd group did not differ significantly and was (66.3±0.81) and (67.5±0.73) years, respectively. We have determined the number of diseases according to medical records data and performed a calculation of multimorbidity indicators – CCI and Combined Age Charlson Comorbidity Index (CA-CCI) [7, 8]. We used statistical software programs (Statistica v. 6.0) and Microsoft Excel 2007 applications for data analysis. 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). Student's t-test was used to compare the mean of a data for the two groups.

RESULTS

According to data of the medical records the number of comorbid diseases in patients having been examined ranged from3 to 12. Ten and more diseases were detected in 17 patients among 60 ones (28.3±5.8%) having undergone urgent PCI and in 34 patients among 78 persons (43.6±5.6%) with planned ones (p=0.06). The majority of patients–46 in the 1st (76.7±5.55%) and 67 in the2nd group (85.9±3.9%) (p=0.18) – had clinical manifestations of angina pectoris (class I or II according to the Canadian Cardiovascular Society grading scale for the classification of angina pectoris severity) [1].We found also both in the 1st and the 2nd groups a high prevalence of arterial hypertension – in 59 patients among 60 ones (98.3±1.6%) and in 78 patients among 78 ones (100%), respectively, cerebrovascular disease [in 45 persons among 60 ones (75±5.6%) and in 69 patients among 78 ones (88.5±3.6%), respectively, p=0.046], atherosclerotic damage of retina vessels [in 39 patients among 60 ones (65±6.2%) and in 52 patients among 78 ones (66.7±5.3%), respectively, p=0.84], peripheral arterial disease [in 19 patients among 60 ones (31.7±6.0%) andin 38persons among 78 ones (48.7±5.6%),respectively, p=0.043], and cardiac rhythm disturbance [in 20 patients among 60 ones (33.3±6.1%) and in 23 patients among 78 ones (29.5±5.2%), respectively, p=0.63]. Heart failure NYHA Class I or II has been diagnosed in all the patients examined. Type 2 diabetes mellitus has been found in 13 patients among 60 ones (21.7±5.3%) of the 1st group and in 27 patients among 78 ones (34.6±5.4%) of the 2nd group (p=0.09); 24 patients (56.7%±6.4%) of the 1st group and 33 ones (42.3±5.6%) of the 2nd group, p=0.09 had impaired glucose metabolism – namely impaired fasting glycaemia with fasting plasma glucose levels ≥ 5.6 mmol/L. Renal disease has been diagnosed in 4 patients among 60 ones of the 1st group (6.7±3.2%) and in 8 patients of the 2nd group (10.3±3.4%), p=0.45.

Chronic cholecystitis and chronic pancreatitis were the most common disorders among digestive tract diseases in both groups: chronic cholecystitis has been registered in 19 patients of the 1st group (31.7±6.0%) and in 28 patients of the 2nd group (35.9±5.4%), p=0.61, chronic pancreatitis – in 11 patients of the 1st group (18.3±5.0%) and in 26 patients of the 2nd group (33.3±5.3%), p=0.04.

Osteochondrosis is found to be a rather frequent disorder of musculoskeletal system being found in 26 patients among 60 ones (43.3±6.4%) in the 1st group and in 42 patients among 78 ones (53.8±5.6%) in the 2nd group, p=0.22. Osteoarthrosis was detected in 10 patients among 60 ones (16.7±4.8%) of the 1st group and in 23 patients among 78 ones (29.5±5.2%) of the 2nd group, p=0.07. Cancer (any malignancy, including malignant neoplasm of the skin) were diagnosed in 9 patients (15.0±4.6%) of the 1st group and in 10 patients (12.8±3.8%) of the 2nd group, p= 0.71.

Only diseases having been included to the index calculator are taken into account for CCI and CA-CCI calculations [7, 8]. The mean number of diseases and the mean number of diseases being taken into account for the CCI calculation and CA-CCI level in patients of both groups are presented in Table I.

DISCUSSION

According to the current guidelines, myocardium revascularization in CCS patients should be followed by efficient management and prevention measures [9, 10]. Comorbidity may have a negative effect on patient`s prognosis due, in particular, to limited possibilities of drug use [1]. Besides, it leads also to worsened quality of life; consequently, the better understanding of concomitant pathology is of great importance for the comprehensive assessment of clinical status of CCS patients following PCI [1, 10].

Our study demonstrates that the number of comorbidity diseases in CCS patients having undergone PCI ranged from 3 to 12. It is necessary to take into account the fact the number of patients with 10 or more comorbidity diseases was 28.3 % in the 1st group and 43.6 % in the 2nd one. Such a fact increases the risk of polypharmacy and requires a very attentive approach concerning the drug therapy. We have found arterial hypertension to be the most common comorbidity in our patients, our result being in accordance with the data of other researches. Among patients having been included to the Ukrainian registry of acute myocardial infarction there are 79% of arterial hypertension cases [11]. The results obtained suggest the necessity to optimize the patient management and monitoring to reach a target level of arterial pressure in all the CCS patients having undergone PCI. Special attention should be paid to the significant spreading of type 2 diabetes mellitus and impaired glucose metabolism. According to current clinical

Table I. The mean number of diseases, CCI, and CA-CCI level in patients with chronic coronary syndromes having undergone percutaneous coronary interventions

Indices	1st group(n=60)	2ndgroup(n=78)	p
Number of diseases (Mean±m)	8.2±0.3	9.3±0.4	NS
Number of diseases (Mean±m) included into the CCI calculation	3.8±0.1	3.9±0.2	NS
CCI	4.2±0.2	4.3±0.2	NS
CA-CCI	7.3±0.3	7.5±0.2	NS

1st group: patients having undergone PCI in acute myocardial infarction;
2nd group: patients having undergone PCI for stable coronary artery diseases;
CCI: Charlson Comorbidity Index;
CA-CCI: Combined Age Charlson Comorbidity Index.

guidelines, the presence of type 2 diabetes mellitus leads to two-fold increase of cardiovascular events in CCS patients [1]. Because of such circumstances, it is important to implement an interdisciplinary approach to achieve control of essential risk factors; such an approach requires not only an improvement of glycaemic control and monitoring of glycated hemoglobin level, but also the monitoring of arterial pressure and low density lipoprotein cholesterol levels in these patients [1].

Our comparative analysis of comorbidity spreading in patients having undergone PCI detects some unimportant differences: among the 2nd group patients there are significantly more oft diagnoses of cerebrovascular disease, peripheral arterial disease, chronic pancreatitis ($p<0.05$) – these features which may be due to long-term chronic process course. No significant differences are found between groups concerning other comorbidities. Among a lot of issues needing their resolution, an important one is a problem of standardized approaches aiming a comprehensive evaluation of patient health for persons with multimorbidity and/or comorbidity and a problem of criteria necessary for evaluation of treating intervention efficiency. For multimorbidity evaluation, several standardized approaches have been proposed taking into account not only the presence of disorders, but also their severity and patient's functional condition. The Charlson Comorbidity Index (CCI) is among the most known ones, it is used from 1987. Several CCI modifications have been proposed, in particular, a modification taking into account the alphanumeric code according to the International statistical classification of diseases and related health problems as well as a CCI variant taking into consideration patient's age – Combined Age CCI (CA-CCI) [7]. It is widely used in cases of longitudinal studies to determine multimorbidity in patients being examined. Taking into consideration current achievements in the management of cardiovascular diseases, there are certain limitations concerning CCI informative value for life span prognosis of such patients. In our study no significant difference concerning the CA-CCI value has been found between patient groups (Table I).

Consequently, the results of our studies prove the high spreading of comorbidities among CCS patients having undergone both urgent and planned PCI, this fact being important for disease prognosis and patient management. Such an aspect of this problem is still poorly investigated and is to be solved.

CONCLUSIONS

Patients with multiple chronic diseases have increasingly become a critical focus for medical staff, and providing effective clinical care for patients with multimorbidity will remain a primary concern for many years to come. Our study demonstrated a significantly high level of comorbidities in CCS patients having undergone PCI. The mean number of diseases in patients of the 1st and 2nd groups was 8.2±0.3 and 9.3±0.4, respectively, the CA-CCI values were 7.3±0.3 and 7.5±0.2, respectively ($p>0.05$). The most prevalent comorbidities were arterial hypertension, cerebrovascular disease, peripheral arterial disease, type 2 diabetes mellitus, and impaired fasting glycaemia. These patients need an interdisciplinary approach to determine the management according to current guidelines aiming to improve outcomes – the decrease of cardiovascular events development and increase of life span.

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ORIGINAL ARTICLE
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ANTIPHOSPHOLIPID AND ANTINEUTROPHIL ANTIBODIES LEVELS IN MEN WITH STABLE CORONARY HEART DISEASE AND POSTINFARCTION CARDIOSCLEROSIS AND ITS RELATIONSHIP WITH THE DISEASE MANIFESTATION

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ABSTRACT

The aim is to study the levels of antiphospholipid (aPL) and antineutrophil antibodies in men with stable coronary heart disease (CHD) with postinfarction cardiosclerosis and to evaluate its relationship with the disease manifestation.

Materials and methods: 164 men with stable CHD and postinfarction cardiosclerosis (53.0 ± 9.14 (M ± σ) years) and 48 age-matched men without CHD were examined. The total aPL IgG and IgM, beta-2 glycoprotein 1 antibodies (anti-β2-GP 1) IgG and IgM, and antibodies for neutrophil proteinase-3 / myeloperoxidase (anti-PR3 / MPO) IgG were determined by ELISA.

Results: Positive levels of aPL and anti-β2-GP 1 of IgG were identified in 56.7% (33.5% double positivity of aPL + anti-β2-GP 1) and 29.2% of control group ($p < 0.001$), while the IgM was lower (11.6% vs. 6.2%, $p = 0.55$, respectively). Significantly higher (1.5-1.7 times) levels of aPL and anti-β2-GP 1 were identified in patients who underwent myocardial infarction (MI) aged less than 44 years, after Q-MI, recurrent MI, in the presence of ischemic stroke, livedo reticularis. In 6.7% of patients with positive levels of aPL and anti-β2-GP 1 low IgG anti-PR3 / MPO levels were detected.

Conclusions: In men with postinfarction cardiosclerosis, IgG positivity according to total aPL and anti-β2-GP 1 is associated with a higher incidence of Q-MI and with recurrent MI. Men with postinfarction cardiosclerosis have a tendency to increase anti-PR3 / MPO levels of IgG under conditions of double aPL positivity and anti-β2-GP1 of IgG.

KEY WORDS: coronary heart disease, antiphospholipid syndrome, antineutrophil antibodies

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INTRODUCTION

Antiphospholipid syndrome (APS) as an independent factor of myocardial infarction (MI) and other forms of coronary heart disease (CHD) is attracting increasing attention [1; 2; 3]. The prevalence of primary APS is 40-50 cases per 100,000 population and annually approximately 5 new cases per 100,000 population are detected [4]. Among patients with acute coronary syndrome, the frequency of APS ranges from 6.1% to 43.3% [5]. The development of APS is due to the formation of antibodies to the own phospholipids (cardiolipin, phosphatidylserine, phosphatidylinositol), cofactor proteins and their complexes with phospholipids (antibodies to β2-glycoprotein 1 (anti-β2-GP1), annexin, thrombomodulin etc). Antiphospholipid antibodies (aPL) cause immune inflammatory activation of the endothelium, initiate the development of thrombophilia, thrombosis and atherothrombosis of various vessels, including coronary arteries, leading to MI [2]. On the other hand, ischemic-reperfusion lesions of the myocardium can act as a trigger for antigen-dependent aPL synthesis and the development of APS, as cardiolipin, a phospholipid with the most potent immunogenic properties enter the circulatory channel [6]. There is evidence in one of the studies that elevated aPL levels of IgG and IgM were detected in 100% of patients in the acute period of MI [7].

Another factor influencing inflammatory activation of the coronary artery endothelium may be antibodies to neutrophil proteinase-3 and myeloperoxidase (anti-PR3 / MPO) [8; 9]. Anti-PR3 / MPO cause leukocyte degranulation and endothelial lining of small vessels (arterioles, venules, capillaries) and cause microcirculatory disorders. Microcirculatory disorders significantly impair myocardial status and increase the risk of CHD progression [10; 11]. It has been recently demonstrated that anti-PR3 / MPOs can be produced concurrently with aPL, which aggravates systemic vasculitis [12]. The question of possible associations of APS components with anti-PR3 / MPO in CHD patients with postinfarction cardiosclerosis remains open.

THE AIM

The aim is to study the levels of aPL and antineutrophil antibodies in men with stable CHD with postinfarction cardiosclerosis and to evaluate its relationship with the disease manifestation.

MATERIALS AND METHODS

An open cross-sectional study was conducted on a case-control basis. 164 patients with stable CHD with

Table I. Clinical and demographic features of CHD group and control group

	Patients with CHD, n=164	Control, n=48	p value
Age, years (M±σ)	53.0±9.14	52.1±8.69	0.860
Body mass index, kg / m2 (M±σ)	29.9±3.74	29.2±2.68	0.141
Body mass index ≥ 30 kg / m2, n (%)	83 (50.6 %)	18 (37.5 %)	0.139
Waist circumference, sm (M±σ)	102.9±9.0	100.5±9.9	0.110
Waist circumference ≥ 94 sm, n (%)	133 (81.1%)	34 (70.8 %)	0.159
AH, n (%)	140 (85.4%)	36 (75.0 %)	0.124
SBP, mm Hg (M±σ)	138.4±15.5	134.7±12.5	0.261
DBP, mm Hg. (M±σ)	86.9±10.1	85.6±10.2	0.592
Smoking, n (%)	67 (40.9 %)	15 (31.3 %)	0.243

Table II. Frequency of detection of phospholipid antibodies IgG class in men with stable CHD with postinfarction cardiosclerosis

Group	Frequency of IgG class antibody levels, n (%)						
	Normal	Low-positive			Medium-positive		
		aPL	anti-β2-GP 1	aPL + anti-β2-GP 1	aPL	anti-β2-GP 1	aPL + anti-β2-GP 1
Control, n=48	34 (70.8%)	6 (12.5 %)	4 (8.3%)	2 (4.2%)	1 (2.1%)	1 (2.1%)	0 (0.0%)
Patients with CHD, n=164	71 (43.3%)	7 (4.3 %)	9 (5.5%)	42 (25.6 %)	11* (6.7%)	13* (7.9%)	11 (6.7%)
p	<0.001	0.078	0.496	<0.001	0.305	0.199	0.073

Note. * - medium-positive levels of one type of antibodies were combined with low-positive levels of another type of antibodies.

Table III. Phospholipid antibody levels in men with stable CHD disease with postinfarction cardiosclerosis

Group	Total aPL, U / ml (M±σ)		Anti-β2-GP 1, U / ml (M±σ)	
	IgG	IgM	IgG	IgM
Control, n=48	6.56±3.67	3.38±2.26	6.70±3.67	3.80±2.91
Patients with CHD, n=164	11.5±5.89	6.14±4.07	12.7±6.74	6.72±3.93
p	<0.001	<0.001	<0.001	<0.001

postinfarction cardiosclerosis, at the age of 53.0 ± 9.14 years and total disease duration 42.0 [14; 99] months were examined. All patients were treated at the cardiology and polyclinic departments of Vinnytsia Regional Clinical Hospital named after Pirogov within 2013-2018. The study was conducted in compliance with bioethic norms according to the Helsinki Declaration "Ethical Principles for Medical Research Involving Human Subjects" with subsequent revisions, European Convention of Human Rights and Biomedicine.

The diagnosis of stable CHD was established according to the recommendations of the AHA / ACC (2014) and ESC (2013). Postinfarction cardiosclerosis was verified on the basis of examination of relevant medical records and the results of instrumental studies. The criteria for inclusion of patients in the study were as follows: male; age > 25 years; verified postinfarction cardiosclerosis; duration of the disease from 3 months or more after the last MI; patient's consent to participate in the study.

The study involved men with postinfarction cardiosclerosis. Exclusion criteria were the following: female, acute coronary syndrome, uncontrolled arterial hypertension (CBP ≥ 180 mm Hg, DBP ≥ 100 mm Hg), hemodynamically unstable arrhythmias, type 1 and 2 diabetes mellitus with decompensated conditions. The study included 123 (75%) patients who underwent Q-MI and 41 (25%) patients who underwent non-Q-MI, including 17 (10.4%) patients who had recurrent MI. Comorbid conditions were found in 143 (87.2%) patients, the most common being arterial hypertension (AH) (85.4%) and abdominal obesity (50.6%). The control group consisted of 48 men aged 52.1 ± 8.69 years and corresponded to the main group by clinical and demographic parameters (Table I).

Blood for the studies was obtained under standard conditions, in the morning on an empty stomach after a night of fasting. The serum was stored in eppendorf microtubes at – 20°C until the study. The determination of total aPL (to cardiolipin, phosphatidylserine, phosphatidylinositol) IgG and IgM was performed by ELISA using the Anti-Phospholipid Screen IgG / IgM kit (Orgentec Diagnostika GmbH, Germany). The results were interpreted as follows: negative result (normal level) – aPL < 10 U / ml, positive result – ≥ 10 U / ml. aPL levels above 40 U / ml were considered high-positive, 20 – 40 U / ml – medium-positive, 10 – 20 U / ml – low-posi-

Table IV. Levels of antibodies to neutrophilic proteinase-3 / myeloperoxidase (anti-PR3 / MPO) IgG class in men with stable CHD with postinfarction cardiosclerosis

Group	Anti-PR3 / MPO IgG, U / ml (M±σ)	Frequency level anti-PR3/MPO, n (%)	
		Negative	Low-positive
Control, n=48	1.81±1.51	48 (100 %)	0 (0.0%)
Patients with CHD, n=164	3.43±2.84***	153 (93.3 %)	11 (6.7 %)
Including those depending on antibodies levels to phospholipid IgG class			
Group 1, n=71	2.78±1.80 *	70 (98.6 %)	1 (1.4 %)
Group 2, n=58	3.39±3.06 **	54 (93.1 %)	4 (6.9 %)
Group 3, n=24	4.76±3.56*** #	20 (83.3 %)	4 (16.7 %)* #
Group 4, n=11	4.91±4.05*** #	9 (81.8 %)	2 (18.2 %)*#

Notes: group 1 – normal levels of aPL and anti-β2-GP 1; group 2 – low-positive levels of aPL and/or anti-β2-GP 1; group 3 – medium-positive levels of aPL or anti-β2-GP 1; group 4 – medium-positive levels of aPL + anti-β2-GP 1.
* – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ compared to control group;
# – $p < 0.05$ – compared to group 1.

Table V. Levels of IgG autoantibodies in patients with CHD with postinfarction cardiosclerosis depending on clinical and demographic features

Feature		Levels IgG, U/ml (M±σ)		
		aPL	anti-β2-GP 1	anti-PR3/MPO
Age at which the 1st MI occurred	< 44 years (n = 41)	16.6±6.10	18.0±7.10	3.46±2.48
	≥ 44 years (n = 123)	9.86±4.77**	11.0±5.65**	3.42±2.96
Duration of the disease	≤ 5 years (n = 107)	11.7±5.93	13.4±7.02	3.34±2.71
	> 5 years (n = 57)	12.6±7.15	13.1±7.84	3.99±3.47
Variant of the 1st MI	Q-IM (n = 123)	12.0±5.51	13.6±6.46	3.39±2.52
	Not Q-IM (n = 41)	9.75±5.81*	10.2±6.85*	3.48±3.40
Number of MI	Single IM (n = 147)	10.9±5.22	12.0±5.68	3.35±2.65
	Recurrent IM (n = 17)	17.1±8.32*	19.2±10.9*	4.31±4.17
Comorbid hypertension	CHD without hypertension (n = 24)	11.8±5.84	12.1±5.07	2.99±1.73
	CHD with hypertension (n = 140)	11.6±6.16	12.9±7.05	3.51±2.98
Vascular comorbidity	without CVD and livedo reticularis (n = 147)	10.8±5.43	12.0±6.31	2.81±1.70
	with CVD + livedo reticularis (n = 17)	18.6±7.40***	19.9±6.96***	8.78±4.64**
Body mass index	<30 kg / m2 (n = 81)	11.4±5.95	12.2±5.86	3.18±2.57
	≥ 30 kg / m2 (n = 83)	11.9±5.84	13.3±7.51	3.67±3.07

Notes. * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$ compared to control group

Table VI. Odds ratio for MI in men with CHD with postinfarction cardiosclerosis depending on the laboratory components of APS

Levels of aPL IgG and anti-β2-GP 1 IgG	Odds ratio, OR (95% CI)		p1	p2
	Q-MI	Recurrent MI		
Negative	1	1	-	-
Positive	2.58 (1.26 – 5.28)	2.52 (0.83-7,67)	0.010	0.067
Including:				
Medium-positive	2.63 (0.98 – 7.18)	4.64 (1.36-15.8)	0.059	0.018
Low-positive	2.53 (1.11-5.77)	1.54 (0.66-5.64)	0.028	0.730

Notes. p1 – authenticity about «1st Q-MI »; p2 – «recurrent MI».

tive. The levels of antibodies to anti-β2-GP 1 of IgG and IgM were determined by the Aeskulisa β2-Glyco-GM kit (AESKU diagnostics, Germany, lot 18200). The results were interpreted as follows: negative result (normal level) – < 12 U / ml, low-positive – 12 – 18 U / ml, positive – > 18 U / ml, including medium-positive – 18 – 40 U / ml, high-positive – above 40 U / ml. The verification of clinical and laboratory components of APS was performed according to the diagnostic

criteria of Sapporo (2006) [13] and the latest EULAR-2019 guidelines [14]. Statistical processing was performed using SPSS Statistics 22.0. Student's t-test was used to estimate the difference between groups in the normal distribution and Mann-Whitney U-test was used for the distribution which is different from normal. The normality of the distribution was checked by Kolmogorov-Smirnov and Shapiro-Wilk criteria. Pearson correlation analysis was used to determine relationships between indicators, using Fisher's exact method when comparing the frequency of changes. The odds ratio (OR), confidence intervals (95% CI) were evaluated. The difference at $p < 0.05$ was considered significant.

RESULTS

It was found (Table II) that negative results on both IgG antibodies (aPL and anti-β2-GP 1) in the control group were found to be 1.63 times more frequent than in patients with CHD ($p < 0.001$). Low positive levels of one type of antibody were found in 20.8% of the control group and in 9.8% of patients with CHD ($p < 0.05$). The combination of low-positive levels of aPL + anti-β2-GP 1 was found in 25.6% of patients with CHD, which was 6.1 times higher than in the control group. In patients with CHD, the presence of medium-positive levels of one of the antibodies (aPL or anti-β2-GP 1) was combined with the presence of low-positive levels of another type of anti-β2-GP 1 antibody, and in 6.7% of patients the medium-positive levels of both antibodies were detected simultaneously. In the control group, low-positive aPL and anti-β2-GP 1 IgM levels were detected in 3 (6.2%) and 4 (8.3%) individuals. Among patients with CHD, low-positive aPL and anti-β2-GP 1 IgM levels were detected in 12 (7.3%) and 15 (9.1%) individuals, with medium-positive levels in 7 (4.3%) and 5 (3, 0%) of individuals, respectively, and differences in control group were not statistically significant. Analysis of the absolute values of aPL and anti-β2-GP 1 IgG and IgM showed (Table III) that in patients with CHD these indicators are significantly higher (1.7-1.8 times, $p < 0.001$) than in the control group. 17 (10.4%) patients with CHD, who were detected with positive levels of aPL and anti-β2-GP 1 IgG and IgM, had had a history of non-coronary vascular manifestations of probable APS, 13 (7.9%) had experienced an ischemic stroke or transient stroke attack, and 7 (4.3%) people had had livedo reticularis including 3 of them with the previous stroke.

Among patients with CHD, 11 (6.7%) individuals with low-positive levels of IgG (Table IV) anti-PR3 / MPO were detected, while in the control group 100% of individuals showed negative levels of these antibodies ($p = 0.073$). Positive anti-PR3 / MPO levels were more commonly found in patients with medium-positive aPL and anti-β2-GP1 IgG levels than in patients with negative and low antibody phospholipid levels. On average, the level of anti-PR3 / MPO IgG in patients with CHD significantly exceeded the control group value (1.8 times), and these differences were amplified with increasing levels of antibodies to phospholipids.

The analysis of the levels of aPL, anti-β2-GP 1 and anti-PR3 / MPO IgG class in patients with CHD, depending on clinical and demographic parameters revealed certain features (Table V). So, significantly higher levels of aPL and anti-β2-GP 1 IgG were found in patients with CHD who underwent their first MI under 44 years (1.68 and 1.63 times respectively), in patients with Q-MI (1,24 and 1.33 times respectively), recurrent MI (1.56 and 1.60 times respectively), in the presence of non-coronary vascular manifestations (1.72 and 1.66 times respectively). Differences in the level of aPL and anti-β2-GP 1 in patients with CHD, depending on the duration of the disease, hypertension and body mass index have not been established. Patients with cerebrovascular disease (CVD), such as stroke or transient ischemic attacks and livedo reticularis, were found to have significantly higher levels of anti-PR3 / MPO IgG (3.12 times) than patients without vascular manifestations. There were no other clinical manifestations for differences in anti-PR3 / MPO IgG levels in patients with CHD.

Thus, in men with postinfarction cardiosclerosis, IgG positivity according to total aPL and anti-β2-GP 1 is associated with a higher incidence of Q-MI (OR 2.58, 95% CI 1.26 – 5.28, $p = 0.01$) and in the presence of medium-positive levels of these autoantibodies with recurrent MI (OR 4.64, 95% CI 1.36-15.8, $p = 0.018$). (Table VI).

DISCUSSION

According to the results of this study, in the primary testing of 164 men with postinfarction cardiosclerosis, the overall frequency of aPL positivity was 56.7%, including two antibodies (aPL + anti-β2-GP 1) – 33.5 %. There is evidence that in individuals with triple aPL positivity during primary testing (lupus anticoagulant + cardiolipin antibodies (aCL) + anti-β2-GP 1), this aPL profile is confirmed after 12 weeks in 98% of cases, in individuals with double aPL- positivity in 88% of cases, and in individuals with isolated aPL positivity in only 40% of cases [15]. The results of long-term prospective studies on asymptomatic carriers of laboratory markers of APS indicate a high risk of thrombotic events in the case of double or triple positivity for aPL, while positivity for one type of antibodies is not associated with an increased risk of thrombosis [16]. A meta-analysis of 11 studies with a total of 2425 patients with CHD (283 individuals with IgG positive aCL) showed an increase in the relative risk of major cardiac events in 12 and 24 months (RR 2.17 and 2,11 respectively). [5]. In IgG aCL-positive patients with "juvenile" CHD (younger than 50 years), the relative risk of recurrent major cardiac events after 12 and 24 months increased more significantly (3.21 and 3.24, respectively) [5].

CONCLUSIONS

Thus, in men with postinfarction cardiosclerosis, double positivity for total aPL and IgG anti-β2-GP 1 is closely associated with the early manifestation of CHD (at the age of 44 years), Q-MI and recurrent MI. Besides, in the case

of double positivity to aPL and anti-β2-GP 1 IgG, there is a tendency to increase the levels of anti-PR3 / MPO IgG class, which is associated with vascular pathology (strokes, livedo reticularis). Therefore, the persistence of the production of aPL and antineutrophil antibodies forms an unfavorable pathogenetic pattern that can significantly modify the course of CHD in men.

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Topic: "Metabolic risk factors, cardiovascular remodeling and functional status of kidneys in patients with cardiovascular pathology. Possibilities of pharmacological correction"

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ORIGINAL ARTICLE
PRACA ORYGINALNA

INVESTIGATION OF THE INFLUENCE OF THROMBOPHILIC GENES POLYMORPHISM, INCLUDING SERPIN 1 (PAI-I), FII, PROTHROMBIN AND ITGB3-B INTEGRIN, ON THE FREQUENCY OF STROKE IN ASSOCIATION WITH CONTROLLABLE RISK FACTORS FOR ITS OCCURRENCE

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ABSTRACT

The aim is the analysis of the relationship between the polymorphism of thrombophilic genes, in particular Serpin 1 (PAI-1), F2-prothrombin and ITGB3-β integrin, and the incidence of stroke, as well as the study of factor effects of this polymorphism in association with controlled risk factors (hypertonic disease, smoking, alcohol consumption, diabetes mellitus, obesity, atrial fibrillation).

Materials and methods: A total of 134 patients were examined (men accounted for 44.8%, women 55.2%, average age 62.5 ± 2.1). The statistical analysis was carried out using the following criteria: χ2-Pearson, Fisher's exact criterion (reversible), Chuprov's coefficient of conjugation and dispersion analysis (alternative complex).

Results: The relationship between the frequency of a specific allele of thrombophilia and the incidence of stroke is absent. The reason for such results can be a significant effect of random factors (hypertension, diabetes ...), a significant variability of risk factors, their different frequency in groups (inter- and intra-group differences), a significant (95%) total effect of these factors.

Conclusions: Identification of biochemical or genetic markers of thrombophilic conditions, including polymorphism of the hemostasis system genes, will significantly increase the possibility of adequate pathogenetic treatment and timely prevention of acute cerebrovascular disorders, especially persons of working age, which has great medical and social importance.

KEY WORDS: stroke, gene, polymorphism, risk factors, thrombophilia

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INTRODUCTION

Among the urgent and priority problems of modern neurology strokes firmly hold the leading position due to its large prevalence, high mortality and disability of population in economically developed countries and large financial costs of treatment and rehabilitation [1, 2]. Every year, more than 20 million cases of stroke are registered in the world, making it the second leading cause of death and the third in terms of disability of adults [3]. Among the neurological diseases, cerebral stroke is one of the most significant medical and social problems in most countries of the world, including in Ukraine [4]. The high level of mortality and disability of people who have suffered a stroke, brings the problem far beyond purely medical issues, makes it socially significant on a national scale, strengthens the negative demographic situation in the country.

In recent decades, many studies have been devoted to molecular genetic analysis of ischemic stroke in different populations [5]. These same studies have shown that in the vast majority of cases (about 90%) ischemic stroke is a multifactorial disease in the development of which, in a variety and complex combinations, lifestyle characteristics, environmental factors and individual genetic characteristics of the organism participate. In recent years, much attention has been paid to the study of the effect of genetic predisposition on the state of the coagulating system of blood [6]. The role of polymorphism in a number of genes has been established, the protein products of which regulate the hemostasis system in the development of vascular pathology [6, 7]. It is obvious that one can not speak of the dominant role of any one gene. Most likely, we are talking about a variety of very diverse genes, the combined effect of which is involved in the formation of the so-called polygenic propensity to stroke [8].

Over the past 20 years in the literature over 100 humoral, pathological, hereditary-constitutional and social and economic factors of the risk of stroke have been described.

The most significant of them – age, arterial hypertension, atherosclerosis, heart rhythm disorders, diabetes mellitus, smoking, alcoholism [9].

According to the World Health Organization, more than 300 risk factors for the development of cerebrovascular diseases have been identified. They can be grouped into four categories:

1) The main managed risk factors (high blood pressure, atherosclerosis, smoking, hypodynamia, excess body weight, diabetes mellitus, malnutrition).

2) Other managed risk factors (social status, emotional strain, alcohol or drug abuse).

3) Unfocused risk factors (sex, age, heredity, ethnicity).

4) "New" risk factors (hyperhomocysteinemia, vasculitis, blood coagulation disorder).

One of the topical issues of modern health care is the elucidation of the molecular genetic foundations of the development of cardiovascular diseases. Due to the extraordinary social and medical significance, one of the main subjects of study is a hemostasis system, the functioning of which is one of the leading risk factors for the development of thrombophilic states, including ischemic stroke.

The genes that can lead to ischemic stroke are categorized as: 1) increasing the influence and manifestation of stroke risk factors; 2) affect vascular reactivity, resistance to ischemia and hypoxia. It should also be noted that these categories are not mutually exclusive [10].

Genetic polymorphism is a persistent, unmanageable risk factor. Genetic factors contribute to the development of hypertension, atherosclerosis, hemorheological disorders, diabetes, which are risk factors for stroke, and individual sensitivity to brain ischemia [11]. In more than 1/3 of the cases, the causes of an ischemic stroke remain unclear, so you should think about the presence of genetic predisposition.

The main cause of ischemic stroke is the cerebral artery thrombosis, and thus studying the polymorphism of the genes responsible for the hemostasis is one of the most urgent tasks.

The studies of structural polymorphisms of genes are of great interest that contribute trombophilic states, linking carriage of certain alleles of polymorphic sites of genes and risk of ischemic stroke and progress to identify individuals at increased genetic risk of stroke, improving prognosis and course of cerebrovascular disease [12,13]. There are many genes, certain alleles associated with an increased risk of cerebrovascular disease.

In addition, the risk of developing ischemic stroke increases not only under the influence of polymorphism involving a pair of nucleotides, but also when combining the alleles of several genes, i.e. has a polygenic hereditary predisposition. Often, there is more than one factor of the prothrombotic state. Hereditary thrombophilia can play an important role in the pathogenesis of ischemic stroke due to synergistic, cumulative effects, hereditary effects and some external factors such as smoking, contraceptive use, diabetes, hyperlipidemia, and hypertension. It should be noted that there are no clear guidelines for the treatment of patients with ischemic stroke in the case of hereditary thrombophilia nowadays [14].

Research of polymorphism of genes as a factor of genetic predisposition to various human diseases opens up new opportunities in identifying risk and choosing optimal therapy for each patient [15]. Detection of genetic determinism of ischemic stroke allows preventably determine the risk factors for its occurrence [16]. However, the contribution of mutational damage to genes coding for blood coagulation factors in increasing the risk of developing ischemic stroke has not been definitely defined for the present.

THE AIM

The aim of the study is the analysis of the relationship between the polymorphism of thrombophilic genes, in particular Serpin 1 (PAI-I), FII-prothrombin and ITGB3-β integrin, and the incidence of stroke, as well as the study of factor effects of this polymorphism in association with controlled risk factors (hypertonic disease, smoking, alcohol consumption, diabetes mellitus, obesity, atrial fibrillation).

MATERIALS AND METHODS

A total of 134 patients were examined in Mukachevo CRH (men accounted for 44.8%, women 55.2%, average age 62.5 ± 2.1 years, urban population 67.2%, rural 32.8 %) for the presence of a certain allele of the following genes in them: Serpin 1 (PAI-I), FII-prothrombin, ITGB3-β integrin. Also, a survey was conducted on the presence of risk factors for stroke: hypertension, smoking, alcohol, obesity, atrial fibrillation, diabetes, stroke in direct relatives.

The study was carried out with the consent of the volunteers, and the methodology met the Helsinki Declaration of 1975 and its review in 1983. All participants of the study got acquainted and signed the form of informed consent, the structure of which corresponded to the officially accepted one.

The statistical analysis was carried out using the following criteria:

1) $\chi 2$-Pearson: tables 2x2 (corrected by Yeats); $\chi 2$-Pearson: mxn (arbitrary tables); Fisher's exact criterion (reversible) – for the analysis of the reliability of the relationship between the incidence of stroke and the presence of a definite allele of thrombophilia [17, 18];
2) Chuprov's coefficient of conjugation – to assess the strength of the relationship between the above phenomena [19];
3) dispersion analysis (alternative complex) – for analysis of the total factor influence of polymorphism gene and risk factors (hypertonic disease, obesity, diabetes, etc.) on the incidence of stroke [20].

For a critical level of certainty $\alpha = 0.05$ was considered.

Statistical processing of the obtained results and data was carried out by using the program of Microsoft Excel 2007 with the introduction into the program of the appropriate algorithms for calculating the statistics used criteria.

RESULTS

Patients were divided into 3 groups (Table I) for further study of the influence of factors on performance indicators:

I group: n = 31, men – 51.6%, women – 48.4%, average age 56.8 ± 4 , 8 years. Group II: n = 30, the proportion of men – 53.3%, women – 46.7%, the average age – 55.8 ± 4.5 years. III group: n = 73, the proportion of men – 38.4%, women – 61.6%, the average age – 58.4 ± 3.0 years. And the group was considered a control group.

In this sample of patients, the polymorphism of the following genes was investigated (Figure 1):

1) FII-prothrombin (factor II blood clotting) FII: 20210 G> A; normal allele G / G; allele of risk G / A, A / A; frequency in the population – 2-5%, inheritance by autosomal dominant type.
2) Serpin 1 (PAI-I) – antagonist of the tissue plasminogen activator PAI-I: -675 5G> 4G; normal allele 5G / 5G; allele risk 5G / 4G, 4G / 4G; the frequency is 5-8%.
3) ITGB3-β integrin (platelet fibrinogen receptor) ITGB3: 1565 T> C; normal allele T / T; allele of risk C / T, C / C; frequency – 20-30%.

According to the results of genetic research, the highest frequency of alleles risk, as well as the variability of the trait, was observed for the Serpin gene 1 – 76.9% (40.3% heterozygous, 36.6% homozygous), the lowest relative to the F2-prothrombin gene – 1.6% .

For the analysis of the relationship between the presence of the pathological allele of thrombophilia and the occurrence of a stroke, conjugacy tables were compiled in various combinations. According to the results of calculations (Table II), it can be concluded that there is no relationship between the polymorphism of the studied genes and the frequency of stroke (p> 0.05), regardless of which type of conjugation table was created and used for further analysis.

Note: grouping vertically for a particular gene; for the coefficient of Chuprov's conjugation the interconnection power was estimated on the Chaddock scale.

To evaluate the effect of the factor (the presence of a certain allele of the thrombophilia gene) on stroke frequency, an analysis of variance (an alternative complex) has been used. For this purpose, a special table was constructed (Table III): horizontally – variants of the Serpin 1 alleles (PAI-1) (factor A), vertically – variants of the alleles of the ITGB3-β gene integrin (factor B), in cells – stroke rate (in the numerator – the number of cases of stroke, in the denominator – the total number of cases).

According to the results of the calculations, the total effect of the polymorphism of the genes SIRPIN 1 (PAI-1) and ITGB3-β integrin on the frequency of stroke is 5%, the effect of uncorrected random factors – 95%. The calculated value of F = 0.73 (P = 0.665). The calculated value of F for factor A is 0.13 (P = 0.878), for factor B – 0.23 (P = 0.795), for a combination of factors A and B – 1.27 (P = 0.286). It follows that the influence of the polymorphism of the above genes alone or their combination on the incidence of stroke is not significant (p> 0.05).

Similar calculations using the alternative complex of dispersion analysis were performed between the polymorphism of the Sperpin 1 (PAI-1) and the ITGB3-β integrin (factor A) genes on the one hand and the frequency of controlled stroke risk factors (hypertonic disease, smoking, alcohol use, obesity, and fibrillation atrium, diabetes mellitus, permanent residence) (Figure 2) in the sample under study (factor B). The polymorphism of the FII-prothrombin gene was not included in the calculations due to the low frequency of the allele risk (1.6%).

According to the statistical analysis, the polymorphism of the genes of thrombophilia alone does not affect the incidence of stroke, but a statistically significant total effect of the polymorphic variants of the Sperpin 1 (PAI-1) and ITGB3-β integrin genes in combination with hypertension, diabetes mellitus and place of residence has been found (Table IV).

DISCUSSION

Characteristics of the studied genes are shown below.

I. Polymorphism of the 20210G> A prothrombin gene (FII). The prothrombin gene is localized on the 11th chromosome (llpll-ql2). It codes the amino acid sequence of the prothrombin protein. Prothrombin, or coagulation factor II, is one of the main components of the blood coagulation system. As a result of its enzymatic cleavage, thrombin is formed. This reaction is the first stage in the formation of a blood clot.

The polymorphism of the 20210G> A prothrombin gene is the most significant and most discussed in the literature. This polymorphous variant of the prothrombin gene is a nucleotide substitution of the guanine (G) nitrogenous base (G) on adenine (A) at position 20210 in the third non-translated region of the gene, which leads, in the case of variant A, to high gene expression and, consequently, to increase the level of prothrombin in 1 , 5-2 times. The excess production of prothrombin due to polymorphism contributes to increased risk of thrombophilia, which is a risk factor for developing myocardial infarction, various thromboses, including thromboembolism of the pulmonary artery, which often has a lethal end. An unfavorable variant of polymorphism (A) is inherited by an autosomal dominant type. This means that an increased risk of thrombophilia occurs even in a heterozygous form of polymorphism (G / A). The presence of the 20210G> A polymorphism of the prothrombin gene in a homozygous or heterozygous form significantly (in 3 and more times, and against the background of smoking – 40 and more times) increases the risk of venous thrombosis, including thrombosis of the vessels of the brain and heart, especially at younger ages [21, 22].

Heterozygous carriers of this polymorphic gene variant are 2-5% of Europeans in the general population and 6.2% of all patients with venous thrombosis [21]. Homozygous variant is extremely rare [23].

Women with non-pregnancy and premature placental abnormalities have mutation in 7-8% of cases [24]. When combined with the Leiden mutation, the risk of thrombosis increases by almost 100 times [25]. The mutation was described in 1996. Its main clinical feature is the constantly high level of prothrombin in blood plasma (87% of carriers have its excess 115%). The polymorphism of the 20210G> A prothrombin gene of younger patients is associated with a high risk of thrombosis not only in the peripheral veins

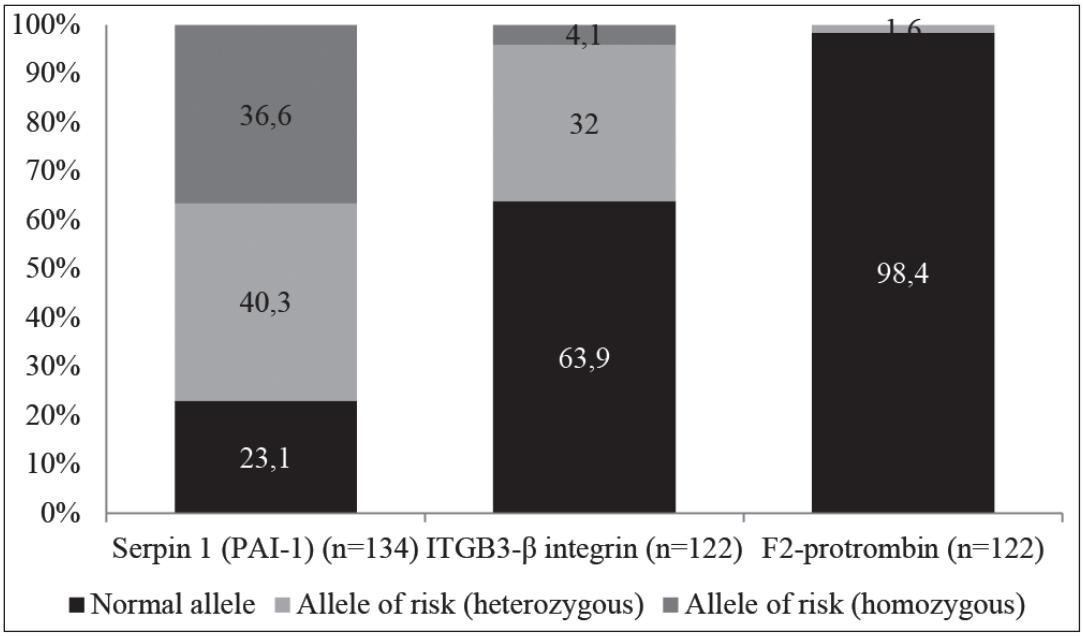

Fig. 1. Frequencies (%) of certain alleles for the whole sample of study.

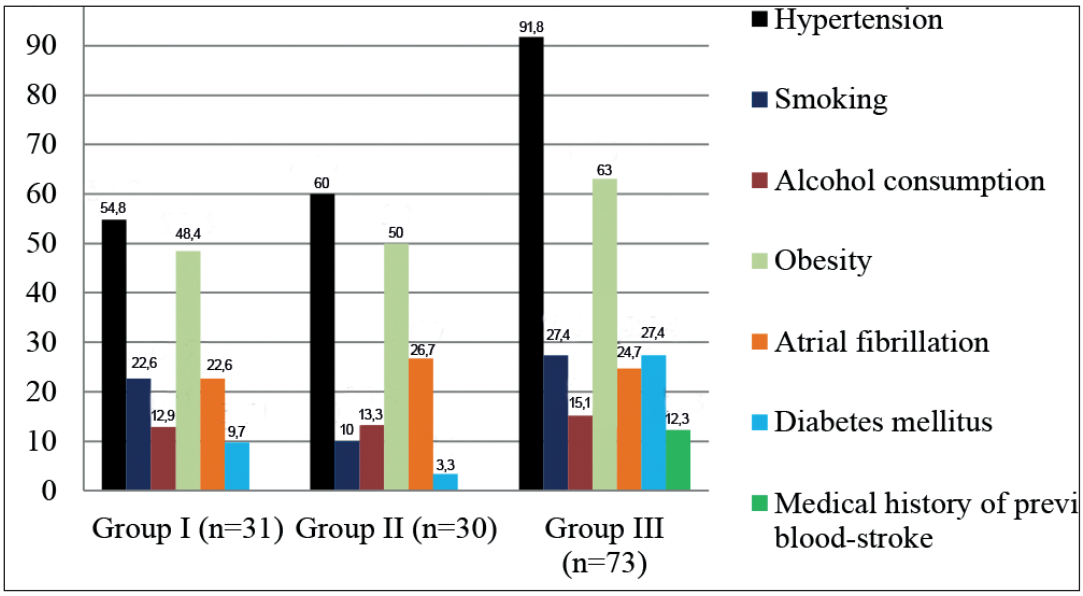

Fig. 2. Specific gravity (%) of people with a risk factor of stroke (in groups).

and veins of the brain, but also in arteries with the development of ischemic stroke and coronary heart disease [26].

II. Polymorphism -675 5G & gt; 4G Plasminogen Activator Inhibitor Type I (PAI-I, SERPINE 1). The basis of the pathogenesis of most thrombophilic states is the decrease in the activity of the fibrinolytic blood system. Therefore, the definition of genetic defects in fibrinolytic potential is an important stage in the diagnosis of hereditary thrombophilia [23].

One of the reasons for the reduction in fibrinolytic activity of the blood is a violation of the conversion of plasminogen into plasmin, due to a decrease in the activity of plasminogen activators. This condition can be either genetically determined, or caused by various acquired states, but most often due to their combination.

Recently, more and more attention has been paid to the role of a type I plasminogen activator (PAI-I) inhibitor in reducing the fibrinolytic potential of plasma.

The type I plasminogen gene is localized on the 7q22.1 chromosome, encoding the protein, an endothelial inhibitor of Plasminogen-1 activator (IAP-1) belonging to the Serpin family. This protein suppresses the work of the tissue plasminogen activator and urokinase, whose function is to activate the conversion of plasminogen to plasma that cleaves fibrin of the blood clots. Thus, PAI-I adversely affects fibrinolysis and prevents dissolution of blood clots, thereby increasing the risk of developing various thromboembolism, including arterial thrombosis.

The basis of the polymorphism of the PAI-I gene is the change in the number of guanine repeats in the promoter region of the gene. There are two variants of the gene with a different number of guanine repeats (G) at positions -675: 5G and 4G. 5G indicates the presence of a sequence of five nitrogen guanine bases. Option 4G indicates the presence of a sequence of four guanine bases – an unfavorable option,

Table I. Distribution of patients by groups

Group	Patients with stroke	Direct relatives with stroke
I	-	-
II	-	+
III	+	+

Table II. Collected data of criterion values of applied statistical methods

Criterion	Serpin 1 (PAI-1)	ITGB3-β integrin	FII- prothrombin
χ2- Pearson: 2x2	0,209 (P=0,648)	0,142 (P=0,706)	0 (P=1)
χ2- Pearson: 2x2 (with Yates' correction)	0,063 (P=0,802)	0,036 (P=0,85)	0,508 (P=0,476)
χ2- Pearson: 2x3	0,738 (P=0,691)	0,482 (P=0,786)	-
χ2- Pearson: 3x3	0,874 (P=0,928)	0,624 (P=0,96)	-
Fischer's exact criterion	P=0,685	P=0,711	P=1
coefficient of Chuprov's conjugation	0,04 (weak interconnection power)	0,034 (weak interconnection power)	0

Table III. The effect of the factor (the presence of a certain allele of the thrombophilia gene) on stroke frequency

Factors B↓ i A→	5G/5G	5G/4G	4G/4G
T/T	a/A	b/B	c/C
C/T	d/D	e/E	f/F
C/C	g/G	h/H	i/I

Table IV. Criteria values and levels of reliability of the dispersion analysis

Gene	Risk factor	F (P)	Share of Total Impact
Serpin 1 (PAI-1)	Hypertension	5,46 (P=0,00014)	17,6%
	Diabetes mellitus	2,41 (P=0,03997)	8,6%
	Place of residence	2,92 (P=0,0157)	10,2%
ITGB3-β integrin	Hypertension	7,59 (P=0,00011)	16,2%
	Diabetes mellitus	3,09 (P=0,029914)	7,3%
	Place of residence	4,22 (P=0,007082)	9,7%

which leads to a decrease in fibrinolysis blood activity due to an increase in plasma concentration of IAP-1. This is due to changes in the processes governing the functioning of the PAI-I gene. When there are five guanine repeats in the promoter region, both activators and transcription suppressors can be contacted with it. Therefore, the regulation of such a gene occurs correctly. In the presence of four repetitions guanine coupling suppressor is violated, therefore, reverse regulation does not occur and the basal activity of protein synthesis is increased. In a homozygote in the 4G allele (4G / 4G genotype), increasing the concentration of IAP-1 in blood plasma leads to an increased risk of thrombocytopenia, and in pregnancy increases the risk of non-pregnancy and to a complication such as pre-eclampsia – it is associated with intervertebral thrombosis or spiral arteries of the placenta.

Timely detection of this allele will allow adequate prevention and avoidance of complications.

The preferred variant of studied polymorphism in the population is the heterozygous variant -675 5G / 4G. In this regard, this polymorphism has no independent diagnostic value, the effect can be evaluated in combination with other factors that lead to the development of pathology (for example, in conjunction with FGB G-455A). The allelic variant -675 4G is accompanied by greater activity of the gene than -675 5G, which results in a higher concentration of PAI-I and a decrease in the activity of the antiglient system of blood [27]. In the in vitro experiment, it was shown that in the 4G / 4G homozygote, the basal level of expression of PAI-I was 25-30% higher than that of the carriers of the 5G allele [7].

According to a number of studies, the 4G / 4G genotype increases the risk of developing thrombosis by an average of 1.7 times. Increasing RAI-1 increases the risk of coronary syndrome and myocardial infarction [28]. Women with the 4G / 4G genotype have an increased risk of developing complications of pregnancy, since suppressed fibrinolysis, which plays an important role in the formation of the mother-placenta-fetus system. What concerns men, this polymorphism increases the risk of coronary thrombosis 5-fold; with coronary artery disease, the 4G / 4G genotype is associated with the development of sudden death. In the presence of variant 4G in the gene PAI-I and C / T in the gene ITGB3-β, the average statistical risk of myocardial infarction increases 4.5 times, and for men – 6 times [29, 30].

Allele 4G occurs in 53-61% of cases in the European population. The prevalence of the homozygous form of this polymorphism in the Europoid populations is 5-8%.

III. Polymorphism of 1565 T> C the fibrinogen platelet receptor gene (ITGB3-β). The gene ITGB3-β encodes the protein component of the fibrinogen platelet receptor, beta-3 integrin (GP IIIa). With the participation of this receptor, the interaction of thrombocytes with fibrinogen, which is contained in the plasma of blood, is carried out. As a result, aggregation of thrombocytes is activated and a thrombus is formed.

This polymorphism is based on the replacement of thymine (T) by cytosine (C) at position 1565 of the gene. As a result, the biochemical properties of the GPIIIa protein change, where the amino acid leucine is replaced by proline at position 59 (Leu59Pro).

The polymorphic variant of the ITGB3-β gene leads to an increase in platelet aggregation, which increases the risk of thrombotic formation and, consequently, the development of cardiovascular pathology, thromboembolism, as well as early termination of pregnancy due to thrombotic lesions of the placenta. The conducted epidemiological studies have shown the association of the polymorphic variant 1565T> C of the gene ITGB3-in with the development of ischemic stroke, as well as coronary heart disease [30]. An increase in the rate of detection of allele C of patients with myocardial infarction under the age of 60 was found to be twice as high as in the control group.

When studying polymorphism of 1565T> C polymorphism of ITGB3-β gene of healthy non-smokers aged 21 to 24 years, it was found that allele C is associated with increased thrombin generation and an antithrombotic effect of aspirin. For patients with T / C and C / C genotypes, the effectiveness of this drug as an antiaggregant is reduced.

In addition, the 1565T> C polymorphism of the ITGB3-β gene is associated with syndromes of thrombocyte immune destruction, to a greater extent with neonatal thrombocytopenia and alloimmune posttransfusion purpura.

Thus, in the development of ischemic stroke, its genetic determinism plays an important role (especially for young people who do not have other most common risk factors).

CONCLUSIONS

Thus, the relationship between the frequency of a certain allele of thrombophilia and the incidence of stroke is absent. The reason for such results can be a significant influence of random factors (hypertension, diabetes, ...), significant variability of risk factors, different frequency in groups (inter- and intra-group difference), a significant (95%) total effect of these factors. The lack of connection in this study may be due to the fact that the pathology of the hemostatic system (thrombophilia) in itself does not significantly affect the incidence of stroke due to the good compensatory mechanisms of the human body for the development of ischemia in this case.

But the significance of the total factor influenceof genes 1 polymorphism (PAI-1) and ITGB3-β integrin and such risk factors as diabetes mellitus and hypertension as well as the place of residence should be noted. Since these controlled factors (arterial hypertension and insulin resistance) are components of a metabolic syndrome, which is a "disease of civilization", and the rate of urbanization only increases (the proportion of urban population in the sample was 67.2%), the study of hereditary pathology of the hemostasis system as unmanaged factor, is rather perverse. It broadens the existing understanding of the heterogeneity of ischemic stroke and consistently confirms the concept of its heterogeneity in mechanisms and causes of occurrence. Identification of biochemical or genetic markers of thrombophilic conditions, including polymorphism of the hemostasis system genes, will significantly increase the possibility of adequate pathogenetic treatment and timely prevention of acute cerebrovascular disorders, especially persons of working age, which has great medical and social importance.

Given that ischemic strokes are multifactorial diseases, in the development of which the complex interaction of various external risk factors plays a part in the presence of a genetic predisposition, the question of which interactions of the acquired and genetic factors, as well as the combination of polymorphic variants of genes lead to the development of arterial thrombosis, determine the features of the course of the acute period of the disease and possible complications, as well as relapses, remains open.

The modern neurologist and scientist need to know the genetic basis of hereditary diseases of the nervous system to a lesser extent than the morphological and physiological patterns of its activity. Further research in the field of medical genetics, neurology and other fundamental disciplines will be the basis of quality treatment and timely prevention of ischemic stroke in combination with thrombophilia.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

DIAPHRAGMATIC BREATHING IN BIOLOGICAL FEEDBACK MODE FOR CORRECTION OF THE PSYCHOPHYSIOLOGICAL STATE IN MEDICAL STUDENTS

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ABSTRACT

The aim: The current study aimed to examine the possibility of correction of the psychophysiological state of the undergraduate students by diaphragmatic breathing sessions in the biological feedback mode utilizing heart rate variability.

Materials and methods: The study enrolled 86 students 18 to 20 years old. Assessment of the functional state of the ANS was performed bycardiointervalography (CIG) and analysis of the spectral indices of HRV by the hardware-software complex "Cardiolab" (KAI Medica, Ukraine). A complex assessment of the autonomic homeostasis was performed using IARS calculated by a special algorithm. Assessment of the psychophysiological state of students was performed by questionnaires.

Results: Among the examined population, 45% of students were in the state of satisfactory adaptation, 43% – in the state of functional tension, and 12% – in the state of unsatisfactory adaptation. Students with poor level of adaptation and depletion of regulatory mechanisms had higher heart rate, systolic and diastolic pressure, and RR compared to the students in the state of satisfactory and strained adaptation.

Conclusions: The findings suggest that the average intensity of psychosomatic complaints has significant differences between groups with varying degrees of tension of regulatory mechanisms.

KEY WORDS: diaphragmatic breathing, biological feedback, psychophysiological state, autonomic nervous system

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INTRODUCTION

A continuous intensification of the educational practices in institutions of higher education is a complex and long-lasting process that significantly affects the psychophysiological parameters of a young person's organism. High levels of psycho-emotional and intellectual tension, increased demands towards the quality of knowledge, and low levels of physical activity all negatively affect the functional capabilities of the students' bodies and result in the stress of the mechanisms of central adaptation [1]. As a result, adaptive reserves are reduced, mechanisms of autonomic regulation are disrupted, and preconditions for the emotional stress and psychosomatic disorders develop [2,3]. The physiological basis of such conditions is the excessive activation of the sympathetic branch of the ANS, inadequate hormonal background, and energy deficit of the cerebral cortex. Therefore, the search for non-pharmacological methods of correcting autonomic imbalances is an urgent medical problem.

To diagnose the functional state of the autonomic nervous system (ANS), the analysis of heart rate variability (HRV) is now actively used in practice [4,5,6]. Another commonly used tool is an integral indicator of the adaptive capacity of the organism – the Indicator of the Activity of Regulatory Systems (IARS), proposed by R.M. Baevsky [7].

Recently, there has been a growing interest in the use of IARS in clinical and restorative medicine [4,8,9], because it allows to characterize not only the degree of stress of the adaptation mechanisms and the initial functional state of the organism, but also its adaptive capacity in changing environmental conditions. Furthermore, it enables comprehensive evaluation of the stressful effects of unfavorable factors on the human body.

Our previous study found a tight relationship between the psychophysiological indicators of medical students and the degree of the ANS tension involving IARS [10]. This allowed us to put forward a working hypothesis that the normalization of the functional state of the ANS in this group of students can cause positive changes in their psychophysiological state.

One of the methods for correction of the functional state of the ANS is the diaphragmatic breathing in the biological feedback mode utilizing heart rate variability.

THE AIM

The study aim was examination of the possibility of correction of the psychophysiological state of the undergraduate

students by diaphragmatic breathing sessions in the biological feedback mode utilizing the heart rate variability.

MATERIALS AND METHODS

The study involved 86 students 18 to 20 years old who did not deviate from the norm according to a physical examination and did not play sports professionally. The studies were conducted in the part of an academic term not involving tests or exams (October-December).

The assessment of the functional state of the ANS was performed by cardiointervalography (CIG) including the analysis of the spectral indices of HRV by the hardware-software complex "Cardiolab" (KAI Medica, Ukraine) (Fig. 1).

Data recording and computer analysis of HRV were performed in accordance with the accepted international standards for analysis of heart rate variability [8,9], as well as according to R.M. Bayevsky [7]. The following HRV spectral parameters were determined: TP, ms^2 – the Total Power of the HRV spectrum; VLF% (Very Low Frequency) – the activity of higher supersegmental centers of autonomic regulation and humoral-metabolic effects; LF% (Low Frequency) – the activity of sympathetic modulators; HF% (High Frequency) – an indicator of vagal influences; LF / HF – sympathetic-vagal balance; IC – centralization index.

A complex assessment of autonomic homeostasis was performed using IARS, which was calculated by a specific algorithm [7].

Based on IARS, there are three functional states of health, which are also called the "traffic light" system: the green zone – a normal state or a state of satisfactory adaptation; the yellow zone – a strain or an extreme strain of adaptation mechanisms; and the red zone – a failure of adaptation.

Assessment of psychophysiological state of students included determination of the level of situational and personal anxiety by the Spielberger-Hanin test method, assessment of the level of stress resistance [11], and investigation of the psychosomatic conditionality of somatic ailments according to the Giesener Beshwedebogen (GBB) questionnaire [11]. The Giesener Beshwedebogen Questionnaire is designed to identify the subjective perception of an individual's physical ailments. Four major and one additional scale were evaluated:

Scale 1. "Exhaustion" (E) – characterizes a non-specific factor of exhaustion, indicating the total loss of vital energy and the need for assistance.

Scale 2. "Gastric complaints" (G) – reflects the syndrome of nervous (psychosomatic) gastric ailments.

Scale 3. "Rheumatic Character" (R) – reflects the patient's subjective suffering of a spastic nature.

Scale 4. "Cardiac Complaints" (C) – indicates that the patient attributes his or her ailments mostly to the cardiovascular area.

Scale 5. "Complaint intensity" or "Pressure" (P) – characterizes the overall intensity of complaints.

Respiratory rate (RR), heart rate (HR) and blood pressure (BP) were measured in all subjects.

The StressEraser device ("Helicor", USA) was used to modulate the functional state of the ANS by diaphragmatic breathing in biological feedback mode with heart rate variability. During the training with the StressEraser, each participant in the experiment adjusted the breathing rate according to the visual signals of the device (Fig.2). The wave structure of the heart rhythm was calculated by the device according to the photoplethysmographic sensor, which detected the pulse of the index finger. The appearance of a triangle marker at the top of the screen signaled the beginning of exhalation. In the case of harmonization of the wave structure of the heart rhythm with the respiratory rhythm, the instrument awarded points to the participant. The session lasted until 30 points were reached, with an average duration of 15-20 minutes during a month.

All obtained digital data were analyzed for differences between groups by Student's t-test at a significance level of $p<0.05$.

RESULTS

Among the examined population, 45% of students were in the state of satisfactory adaptation, 43% were in the state of functional strain, and 12% were in the state of unsatisfactory adaptation (Fig. 3 Distribution of students by IARS).

In the next stage of the study, students with different adaptive capacities were evaluated for a number of psychophysiological parameters (Table I).

Students with the unsatisfactory level of adaptation and depletion of regulatory mechanisms had higher heart rate, systolic and diastolic pressure, and RR compared to students in the state of satisfactory and strained adaptation. Students with unsatisfactory levels of adaptive capacity had a higher level of personal anxiety and low levels of stress resistance compared to the group of satisfactory adaptation.

Analysis of the results of the intensity of psychosomatic ailments according to the Giesener Beshwedebogen questionnaire (Table II) showed that significantly higher mean values of psychosomatic ailments on all scales were found in the group of students with unsatisfactory adaptation.

The results obtained in this study show that the average intensity of psychosomatic complaints is significantly different between groups with varying degrees of tension of the regulatory mechanisms. Thus, the mean values of psychosomatic complaints were significantly higher in individuals with unsatisfactory adaptation on all scales, with $p<0.05$ on the "Gastric", "Rheumatic", and "Cardiac" scales and $p<0.01$ on the "Exhaustion" and Pressure" scale.

In the next phase of our study, a course of diaphragmatic breathing sessions was conducted in the biological feedback mode with heart rate variability using the StressEraser device (Helicor ", USA) in order to optimize the functional state of the ANS in students with the strain of regulatory mechanisms (group 1) and failure of adaptation (group 2).

Dynamics of HRV indicators in the examined groups after the 30-day course of breathing exercises is presented in Table III. After completing the course, a redistribution of activity of the peripheral ANS in favor of a significant increase in HF was noted in two groups. Thus, the relative contribution of the high-frequency part of the spectrum

Figure 1. Computer processing of the rhythmocardiogram of student B. using the Cardiolab device.

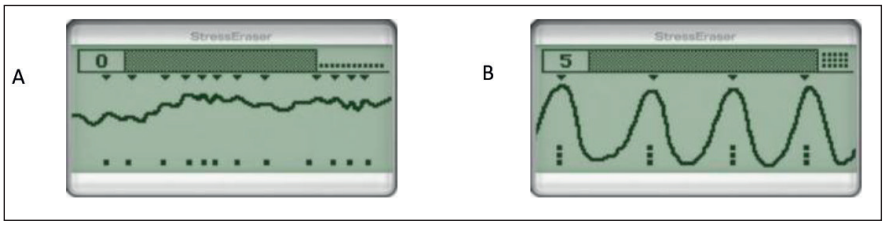

Figure. 2. Visualization of breathing exercises on the StressEraser display
a - during the 1st minute of training; b- during the 15th minute of training.

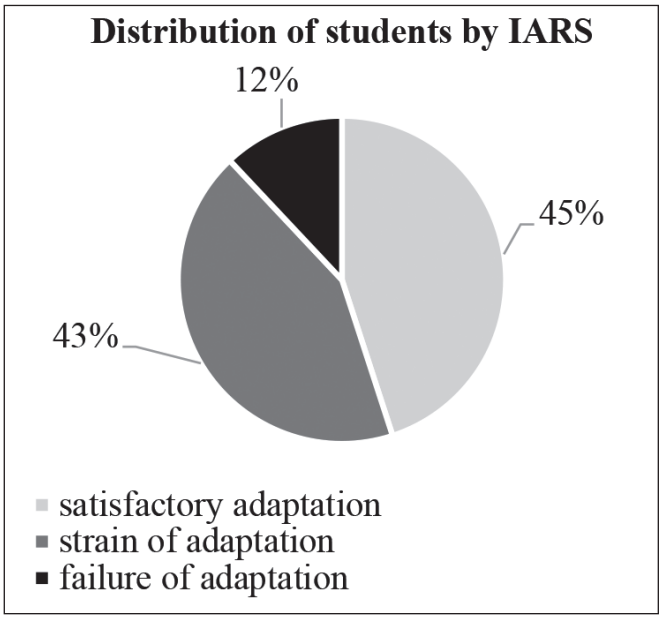

Figure 3. Distribution of students by IARS.

(HF%) to the total heart rate variability increased by 13.3% ($p < 0.01$) and 13.6% ($p < 0.01$), respectively.

A repeat analysis of the results of the intensity of psychosomatic ailments according to the Giesener Beshwedebogen questionnaire after a 30-day course of respiratory gymnastics revealed a significant decrease in the average values of psychosomatic ailments on most scales in two groups of students (Table IV). Thus, the largest change was noted on the "Exhaustion" scale – from 8.8 ± 2.1 to 5.4 ± 0.5 ($p<0.01$) in the first group and from 12.5 ± 2.5 to 6.3 ± 0.9 ($p<0.01$) in the second group. Another positive result was a decrease in personal anxiety, which is considered an integral component of adaptive self-regulation.

DISCUSSION

Personal anxiety is considered to be a stable individual feature of a person that characterizes one's tendency to perceive a certain range of indifferent situations as threatening and dangerous to self-esteem. Highly anxious individuals tend to perceive the threat to self-esteem and vitality and respond with pronounced anxiety. Increased anxiety is the main mechanism of non-adaptive behavior. However, a certain level of anxiety is a natural and a necessary feature of human productive activity. Self-control and self-evaluation of this condition is an essential component of adaptive self-regulation, since heightened anxiety is the leading "obligatory mechanism" of maladaptive disorders.

After a course of diaphragmatic breathing in the biological feedback mode a redistribution of activity of the peripheral ANS in favor of a significant increase in HF was noted in two groups. According to a number of authors,

Table I. Psychophysiological parameters depending on the functional state of students' regulatory systems (M ± m)

Indicators	Satisfactory adaptation (n=45%) 1	Strain of adaptation (n=43%) 2	Failure of adaptation (n=12%) 3
HR. beats per minute.	74±2.3	83±3.1	95±6.5**
SAP. mmHg	119.54±3.3	125±4.3	134.±5.3*
DAP. mmHg	72.7±1.4	79.4±2.5	84.3±4.5*
RR. per minute.	15.2±0.7	17.2±0.9	19.7±1.9*
Personal anxiety	31.7±4.4	46.1±4.2	52.7.2±5.3**
Stress resistance	18±3.7	32±4.3	47±4.8**

Notes: * - probability of the difference in indicators between groups 1 and 3, p<0.05; ** - probability of the difference in indicators between groups 1 and 3, p <0.01.

Table II. Intensity of psychosomatic complaints in students depending on the functional state of regulatory systems (M±m)

Indicators	Satisfactory adaptation (n=45%) 1	Strain of adaptation (n=43%) 2	Failure of adaptation (n=12%) 3
Exhaustion	5.3±0.6	8.8±2.1	12.5±2.5**
Gastric complaints	1.8±0.3	2.7±1.3	4.7±1.3*
Rheumatic complaints	4.4±1.4	5.5±1.5	8.7±3.5*
Cardiac complaints	2.4±0.7	2.9±0.9	5.5±1.9*
Pressure	11.7±3.4	21.1±4.2	32.2±4.3**

Notes: * - probability of the difference in indicators between groups 1 and 3, p<0.05; ** - probability of the difference in indicators between groups 1 and 3, p <0.01.

Table III. Dynamics of HRV indicators in the study groups

Indicator	The first group (strained adaptation) (n=37)		The second group (failure of adaptation) (n=10)	
	Baseline	After a training course	Baseline	After a training course
TP. ms^2	3963.9±474.1	4215±363	1727.9±746.9	2423.9±525
HF. ms^2	1073±55.8	1687±134*	244.2±63.8	673.3±84*
HF. %	27.1±1.3	40.4±2.16**	14.1±3.3	27.7±3.1**
IARS	5.9±1.4	4.2±1.6*	8.8±0.6	5.4±1.2**

Notes: * - statistically significant change from baseline (p <0.05), ** (p <0.01)

Table IV. Dynamics of psychosomatic complaints intensity and the level of personal anxiety in students

Indicator	The first group (strained adaptation) (n=37)		The second group (failure of adaptation) (n=10)	
	Baseline	After a training course	Baseline	After a training course
Exhaustion	8.8±2.1	5.4±0.5**	12.5±2.5	6.3±0.9**
Gastric	2.7±1.3	1.7±0.3	4.7±1.3	2.8±0.5*
Rheumatic	5.5±1.5	4.5±1.5	8.7±3.5	5.1±1.4
Cardiac	2.9±0.9	2.1±0.5	5.5±1.9	2.3±0.7*
Pressure	21.1±4.2	12.8±2.6*	32.2±4.3	15.6±3.6*
Personal anxiety	46.1±4.2	33.4±3.1*	52.7.2±5.3	38.7±4.3*

Notes: * - statistically significant change from baseline (p <0.05), ** (p <0.01)

such restructuring of the autonomous regulation creates a favorable background for modulating psychophysiological responses to mental stressors [7,9,10].

A repeat analysis of the results of the intensity of psychosomatic ailments according to the Giesener Beshwedebogen questionnaire after a 30-day course of diaphragmatic breathing in the biological

feedback mode showed a significant decrease in the average values of psychosomatic ailments on most scales in two groups of students. These results suggest that the use of controlled breathing in the biological feedback mode to increase the variability of heart rate, and its high-frequency components in particular, leads to the optimization of the reflex response to mental stressors and is sufficiently sound physiologically. This method is based on the use of physiological mechanisms of sinus respiratory arrhythmia. It leads to a more complete harmonization of the respiratory cycles with the wave structure of the heart rhythm due to the ability to observe one's own HRV curve and to correct the frequency and depth of breathing. Respiratory training in the biological feedback mode with cardiac activity leads to a significant increase in HF in students with strain and failure of regulatory mechanisms' adaptation and regression of psychosomatic manifestations.

CONCLUSIONS

1. Investigation of the HRV indices in the conditions of normal educational load among students revealed different functional states according to the integrated activity index of the regulatory systems. Depending on the functional reserves, the regulatory systems operated with varying degrees of strain from optimal level to failure. As the strain of adaptation mechanisms increased, the activity of sympathetic influences was enhanced and the influence of respiratory periodicity was reduced.
2. It was established that students with an unsatisfactory level of adaptation and depletion of regulatory mechanisms had significantly higher levels of personal anxiety, low stress resistance, and an increased level of psychosomatic manifestations.
3. Deep breathing in biological feedback mode with heart rate variability shifted the sympathetic-vagal balance towards the parasympathetic branch of the autonomic nervous system and created the optimal background to optimize the response to mental stress.
4. Respiratory gymnastics using portable biological feedback devices can be used to prevent psycho-emotional stress in the education of medical students.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

THE USE OF PHOTODYNAMIC THERAPY IN THE TREATMENT OF DENTAL CARIES IN CHILDREN OF CONTAMINATED AREAS OF THE ECOSYSTEM OF THE UPPER TYSA REGION

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ABSTRACT

The aim: improving the effectiveness of treatment of dental caries in children using the method of photodynamic therapy.

Materials and methods: The treatment of permanent tooth decay was performed in 35 children of the Upper Tysa region aged 12-15 years. Using the polymerase chain reaction method, the clinical efficacy of photodynamic therapy in the treatment of dentin caries was determined.

Results: During the study, by PCR analysis, dentin caries most commonly revealed genetic markers of DNA of the five most virulent anaerobic bacteria: Prevotella intermedia, Fusobacterium spp., Enterococcus Faecalis, Veilonella spp., Candida albicans. After treatment of the carious cavity by the method of photodynamic therapy with exposure of 30 s – fusobacterium spp. were not detected, the detection rate of prevotella intermedia decreased 3-fold, enterococcus faecalis 3.5-fold, veilonella spp.- 5-fold, and candida albicans- 8-fold; after treatment with carious cavities with 60 s exposure – anaerobic microorganisms were not detected in the investigated samples. The effect of photodynamic therapy with laser exposure of 60 seconds on caries-causing streptococci resulted in their total death, and with exposure of 30 seconds – the frequency of isolated strains decreased several folds.

Conclusions: The use of photodynamic therapy in the treatment of dentine caries is a highly effective and pathogenetically sound method of treatment that provides a significant reduction in the optional and obligate types of cariesogenic microorganisms.

KEY WORDS: laser FotoSan 630, PDT (photodynamic therapy), PCR (polymerase chain reaction), caries

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INTRODUCTION

The relevance, prevalence, effectiveness and prevention of caries and its complications continue to be at the forefront of dental health issues in Eastern Europe, including Ukraine. According to WHO, dental caries in most countries of the world range from 80% to 98% and progress to 100% in low-living countries [1]. Analysis of the structure of carious lesions in children of 15-18 years living in the combined negative effects of factors of natural-technological genesis (Upper Potysia region), according to the ICDAS II system, shows that in children of low biogeochemical zone the caries prevalence is lower, unlike children and the mountain zone where carious cavities on the proximal surfaces are much more prevalent, and the tendency to increase the number of carious cavities is even more pronounced [2]. Despite some advances in the treatment of dental caries, the search for new treatments and treatments remains relevant. Recently, work has emerged on the feasibility of incorporating photodynamic therapy (PDT) as a new strategic direction for the stage of secondary prevention of caries. The use of PDT can be attributed not only to its effect on the vast majority of pathogenic bacteria in the microbial landscape of carious cavities, but also to the activation of microcirculation in the pulp as a result of regulatory action on the microvasculature by laser radiation [3]. The essence of the photodynamic reaction is the formation of free radicals or short-lived forms of singlet (active) oxygen. In terms of photodynamic reactions, it should be noted that singlet oxygen and free radicals are short-lived forms and are inactivated for one millionths of a second, decaying into their original components and thus not posing a risk to other cells [3]. The most important feature of the interaction of biological tissues with molecules of photosensitizing substance is the selective consumption of it only "harmful" to the body cells, which allows to maintain normally functioning and destroy atypical, which is an obstacle to the activity of the whole organism. The type of such "harmful" cells is determined by the hyperactivity of their metabolic activity, which exceeds the normative level of cellular functioning [3, 4]. The level of dental health in children is closely linked to an increase in the relative weight of the risk factors for the formation and progression of diseases of hard and soft tissues of the oral cavity, which is reflected in the structure of the main dental diseases[1].

Table I. The distribution of patients into groups according to the method of antiseptic treatment of carious cavity.

Groups	Method of antiseptic treatment of carious cavity	Number of patients	Number of teeth
№1	Photodynamic therapy: treatment with photosensitizer followed by laser irradiation for 30 s	12	15
№2	Photodynamic therapy: treatment with photosensitizer followed by laser irradiation for 60 s	12	17
№3 (control group)	Traditional treatment: washing with 0.12% solution of chlorhexidine	11	14
Total		35	46

Transcarpathia refers to the climate-geographic zone with a low level of fluoride and iodine in the environment, and as shown by epidemiological surveys «very high» according to WHO criteria, the level of intensity of major dental diseases associated with a deficit in the daily intake of iodine and fluorine [2]. In the domestic and foreign literature there are a sufficient number of publications confirming the clinical effectiveness of the use of photodynamic therapy in dentistry in diseases of periodontal tissues, endodontic treatment, as well as obtaining solutions for irrigation of the oral cavity [5,6,7,8,9]. In recent years, at the same time photoactivating disinfection has been used in dentistry in the treatment of caries and its complications, in periodontics, implantology, in pathologies of the mucous membrane, in maxillofacial surgery [10]. Publications on the use of photodynamic therapy in the treatment of dental caries are isolated [11], some of them are not scientifically substantiated. In this regard, conducting a study to study the effectiveness of the use of photodynamic therapy in this pathology is relevant today.

THE AIM

The purpose was to improve the clinical efficacy of standard dental caries treatment protocols in children of the Upper Tysa region by using photodynamic therapy.

MATERIALS AND METHODS

The clinical study was conducted on the basis of the dental department of the Central District Hospital in Berehovo. There were 35 patients aged 12 to 15 years under clinical observation who were treated for 46 permanent bite teeth; with dentine caries, under the control and written consent of the parents. When making a diagnosis of caries used the conventional classification International Statistical Classification of Diseases and Related Health Problems. The study is based on the main provisions of the GCP ICH and the Helsinki Declaration on Biomedical Research, the Council of Europe Convention on Human Rights and Biomedicine (2007) and the recommendations of the Committee on Bioethics at the Presidium of the National Academy of Sciences of Ukraine (2002). Violations of moral and ethical standards were not found during the study. Tested laboratories are certified. Depending on the method of antiseptic treatment of caries, all patients included in the study were divided into 3 groups (table I). In groups №1 and №2, tooth treatment was performed using photodynamic therapy, in group №3 (control) – traditional antiseptic treatment of carious cavity with 0.12% solution of chlorhexidine. The algorithm of photodynamic therapy was as follows. After the examination and diagnosis, the patient was anesthetized and dissected carious cavity with a turbine tip with the maximum observance of all rules of aseptic and antiseptic. The machining of the hard tissues of the tooth was carried out with cooling due to the constant supply of air and water, in the form of an aerosol, into the treated cavity. After completion of mechanical treatment and isolation of the tooth from saliva in patients of groups №1 and №2 carried out disinfection of the carious cavity by the method of photodynamic therapy. The walls and bottom of the prepared cavity were covered with a photosensitizer FotoSan Agent (toluidine blue) with a uniform layer up to 1 mm, which remained for 30 seconds. Then, irradiation was performed using the FotoSan 630 laser apparatus (CMS Dental, Denmark) in continuous mode, the exposure time was in the group №1 – 30 s, in the group №2 – 60 s. In group No. 3 (control), after the carious cavity preparation, traditional antiseptic treatment with 0.12% chlorhexidine solution was carried out. Further treatment of the teeth in patients in all groups was performed according to the standard technique using adhesive technique and permanent restoration. The sampling of the material was performed by scraping dentin from the walls of the carious cavity with a sterile dental excavator №2. The re-taking of the material was carried out in groups №1 and №2 after photodynamic therapy, after treatment of the cavity with a solution of chlorhexidine. Thus, two groups of dentine sawdust samples taken before and after antiseptic caries treatment were obtained. The obtained dentine sawdust samples were placed in an Eppendorf-type tube containing 500 μl of saline solution, stirred and sent to the AstraDia Microbiology Laboratory. A molecular genetic method for the study of anaerobic microflora – PCR diagnostics in Real time stomatoflora (DK 021: 2015: 33696500-0 UA) was used to detect bacterial DNA marker fragments in the material. The results of laboratory and clinical studies were processed by the methods of variational statistics with determination of the average value, its errors, the Student's t test for multiple comparisons, using Excel (MS Office 2010, Microsoft, USA) and STATISTICA 6.0 (StatSoft, USA). Differences of indicators at significance level $p < 0.05$ were considered statistically significant.

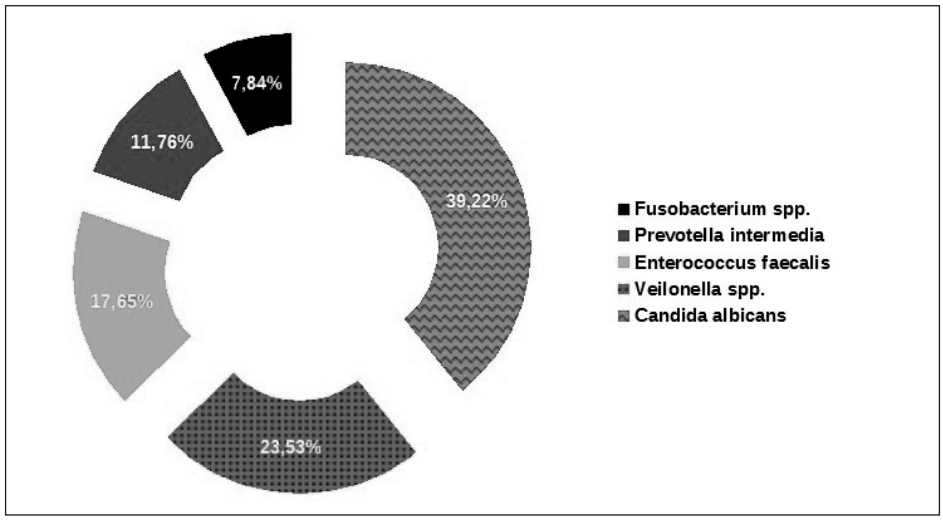

Fig. 1. Comparative PCR detection rate of virulent anaerobic bacteria in dentine caries (n=51)

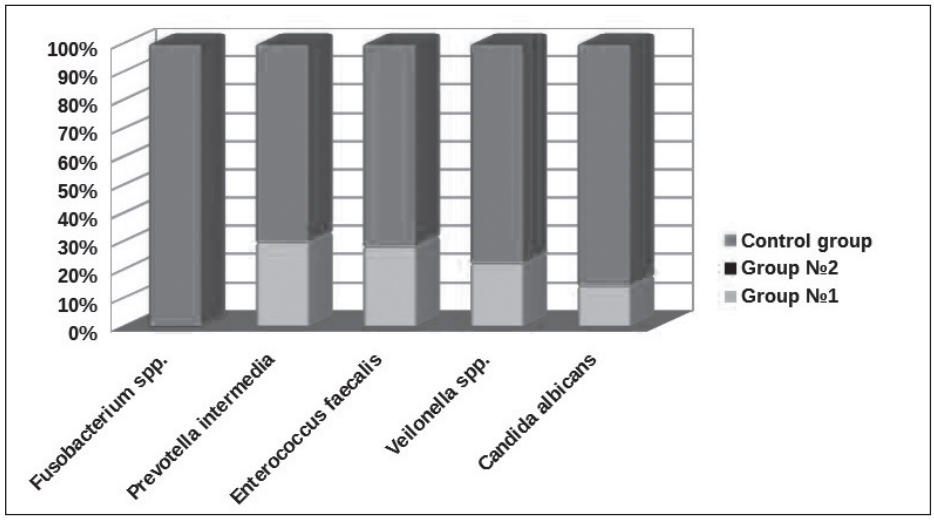

Fig. 2. Comparative frequency of PCR detection of virulent anaerobic bacteria in dental caries after photodynamic therapy.

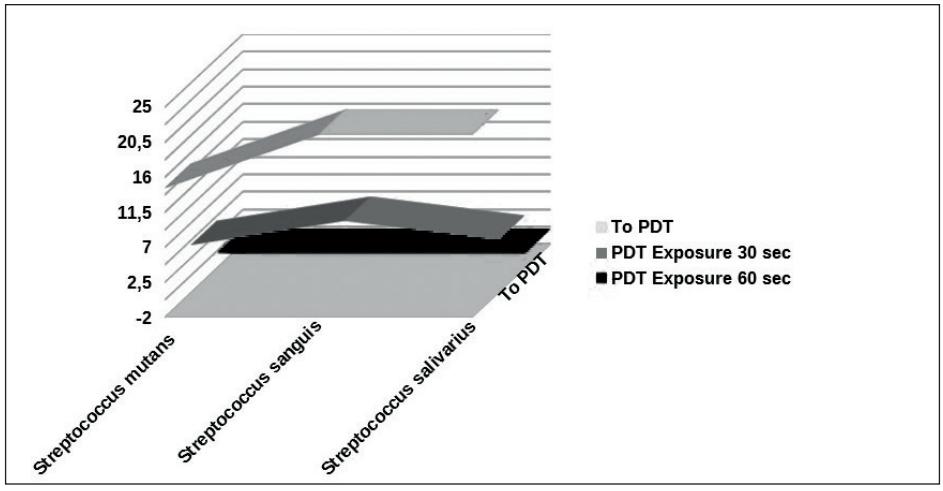

Fig. 3. Frequency of cariesogenic streptococci secretion after photodynamic therapy with exposures of 30 and 60 seconds.

RESULTS

Based on the analysis of the polymerase chain reaction of the microflora associated with the development of dental caries, it was found that pigment-forming DNA was detected in 27 (77.1%) patients. In this case, 7 (21.5%) patients had two types, and 20 (55.6%) had one type of microorganisms. Only 8 (22.9%) patients had no DNA sample of the microorganisms. Such a difference in the average quantitative indicator of the contamination of microorganisms with carious cavity, in our opinion, is explained by the different clinical course of caries. In the course of PCR analysis with dentine caries, genetic markers of the five types of the most virulent anaerobic bacteria were most commonly detected: Prevotella intermedia in 4 (7.84%) cases, Fusobacterium

Table II. Frequency of discharge of carious streptococci in dentine caries

Kind of microorganisms	Selection frequency strains	The titer of the selected strains
Streptococcus mutans	14,64±0,08(p<0,04)	10^5
Streptococcus sanguis	21,53±0,06(p<0,04)	10^6-10^7
Streptococcus salivarius	21,53±0,06(p<0,04)	10^6-10^7

Table III. Frequency of cariesogenic streptococci excretion in dentine caries after administration of 0.12% solution of chlorhexedine.

Kind of microorganisms	Selection frequency strains	The titer of the selected strains
Streptococcus mutans	9,08±0,06(p<0,05)	10^5
Streptococcus sanguis	13,42±0,09(p<0,05)	10^5
Streptococcus salivarius	11,56±0,04(p<0,05)	10^5

spp.-in 6 (11.76%), Enterococcus Faecalis- in 9 (17.65%), Veilonella spp.-in 12 (23.53%), Candida albicans-in 20 (39.22%) cases (Fig. 1). In a clinical study in group №1 after caries treatment with photodynamic therapy with 30 s exposure – Fusobacterium spp. were not detected, the percentage of detection of Prevotella intermedia was 3.92%, Enterococcus faecalis – 5.04%, Veilonella spp.-4.71%, and Candida albicans- 4.9%. In group №2, where caries were treated by photodynamic therapy with 60 s exposure – anaerobic microorganisms were not detected in the studied samples. In group №3 (control), after treatment of the carious cavity with a solution of chlorhexidine, the detection rate of bacteria Prevotella intermedia decreased, compared with the original level, by 9.34%; Fusobacterium spp – 6.31%; Enterococcus faecalis – 12.9%; Veilonella spp. -on 16.62%; Candida albicans at 29.76%. (Fig. 2).

After contaminating carious cavities with bacteria and incubation, teeth were divided into a control group and a test group. Half of the teeth did not under go any intervention and served as the control, where as in the test group the teeth received a solutionof 0.0125 % toluidine blue for 5 min followed by irradiationusing a 50-mW diode laser (Ga–Al–As) at a wave length of 660 nm. Bacterial samples were taken before and after irradiation. The number of colony-forming units was counted and it was concluded that PDT was effective in E.faecalis contamnated carious cavities. These indicators are significantly inferior to those of groups №1 and №2. Thus, after the treatment of carious cavity by photodynamic therapy, the frequency of detection of pathogenic anaerobes was significantly lower than after traditional treatment with a solution of chlorhexidine. The results of the study strongly demonstrate that the technique of photoactivating disinfection successfully destroys anaerobic bacteria with the right combination of the photosensitizer FotoSan Agent (toluidine blue) and an adequate dose of the energy of the red diode laser FotoSan 630 (CMS Dental, Denmark). To determine the antibacterial efficacy of photodynamic therapy for cariesogenic streptococci, we conducted a study of seeding frequency and quantitative ratio of streptococci to caries. In a background study, it was found that Streptococcus sanguis, Streptococcus salivarius were isolated from all carious cavities (100%), and Streptococcus mutans in 68% of cases (Table II).

The effect of photodynamic therapy with laser exposure of 60 seconds on caries-causing streptococci resulted in their total death, and with exposure of 30 seconds – the frequency of isolated strains of Streptococcus mutans decreased from 14,64±0,08 x 10^5(p<0,04) to 4,18 ±0,06 x 10^3(p<0,04), Streptococcus sanguis — from 21,53±0,06(p<0,04) x 10^6-10^7 to 7,28±0,09(p<0,04) x 10^3-10^4, and Streptococcus salivarius — from 21,53±0,06(p<0,04) x 10^6-10^7 to 4,84±0,07(p<0,05) x 10^3(Fig.3).

In the control group, where a 0.12% solution of chlorhexedine was used as an antiseptic for the treatment of carious cavity, there was also a decrease in the frequency of discharge and the number of streptococci, but less pronounced than in patients groups 1 and 2 (Table III).

The results of microbiological studies indicate that the use of photodynamic therapy in the treatment of patients with various forms of caries is a highly effective and pathogenetically sound treatment that provides a significant reduction of optional and obligate types of cariesogenic microorganisms. Based on the study, it can be considered that photodynamic therapy has a more effective effect on infected hard tissues of the tooth, which contributes to a more complete elimination of microorganisms, improve the tight fit of the filling material and prevent the development of secondary caries. Given that the origin and development of caries infectious factor is crucial, the relevance of the study of aspects of photodynamic therapy in this application, in our opinion, is not in doubt.

DISCUSSION

Fonseca et al. [12] have investigated the effects of antimicrobial photodynamic therapy on cariesogenic pathogens by evaluating the decrease in numbers of Enterococcus faecalis colonies in the carious cavities of extracted human teeth. During the clinical study Velichko I. after a carious cavity by photodynamic therapy with laser light irradiation for 30 s percent detection of Veilonella spp. 4.7-fold Enterococcus faecalis – 2.-fold, Candida albicans 6.8-fold, Fusobacterium spp.-fold [11]. Biofilms of Streptococcus intermedius, Streptococcus mutans, Streptococcus salivarius prepared in carious cavities extracted human teeth, have been subjected to photodynamic antimicrobial chemotherapy using

toluidine blue O, a laser diode device emitting at 633 nm. Photoactive disinfection significantly reduced the number of bacteria in repaired dental cavities: the quantitative value of Streptococcus intermedius decreased by 87%, Streptococcus mutans – by 71%, Streptococcus salivarius – by 82%. [4]. The results of the Pierre Adriano Moreno NEVES study showed a statistically significant difference in the number of viable microorganisms before and after PDT application in molars that had been removed for partial removal of carious tissue. Therapy resulted in an average log decrease of 0.61 in the total number of microorganisms, 0.44 in streptococcal mutants and 0.46in Lactobacillus spp. ($p > 0.05$) [13]. However, this therapy presents different challenges on the susceptibility of different microorganisms. Most of the photosensitizers used in PDT are significantly more effective in inactivating Gram-positive bacteria than Gram-negative bacteria, which favors their use against dental caries microorganisms, since these caries lesions typically present the prevalence of Gram-positive strains [14].

CONCLUSIONS

In the study of microbial contamination of the carious cavity with pathogenic flora by PCR diagnostics, the genetic markers of the DNA of the five most virulent anaerobic bacteria were most commonly detected: Prevotella intermedia in 4 (7.84%) cases, Fusobacterium spp.-in 6 (11.76%) %), Enterococcus Faecalis- in 9 (17.65%), Veilonella spp.-in 12 (23.53%), Candida albicans-in 20 (39.22%) cases. When exposed to the red diode laser FotoSan 630 on the microflora of carious cavities of children of the Upper Tysa region with an exposure of 30 seconds, there was a decrease of pathogenic flora in carious cavities by 40%.

The maximum antiseptic effect of treatment of carious cavities was observed with the use of photodynamic therapy with an exposure time of 60 seconds, in which total destruction of pathogenic microflora occurred. The least effective method of antiseptic treatment is the use of 0.12% chlorhexidine, in which the reduction of pathogenic flora occurred by only 20%. The impact of photodynamic therapy with laser exposure for 60 seconds on caries-causing streptococci resulted in their total death. Given that in the pathogenesis of caries infectious factor is crucial, the relevance of the study of aspects of photodynamic therapy may in the near future be a real alternative to traditional methods of antibacterial exposure.

The obtained experimental and clinical data allow us to confirm the possibility and effectiveness of the use of photodynamic therapy in the clinic of therapeutic dentistry, as a new strategic direction of secondary prevention of carious disease.

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Conflict of interest statement
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ORIGINAL ARTICLE
PRACA ORYGINALNA

CLINICAL SYNDROMES OF THE THALAMIC STROKE IN THE CLASSICAL VASCULAR TERRITORIES: A PROSPECTIVE HOSPITAL-BASED COHORT STUDY

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ABSTRACT

The aim: We aimed to determine, describe, and analyze the clinical and neuroimaging features of vascular syndromes of acute thalamic stroke in the classical vascular territories in a prospective hospital-based cohort study.

Materials and methods: We have prospectively recruited 319 acute stroke patients, admitted to the Neurological Center at an academic hospital (Oleksandrivska Clinical Hospital) in Kyiv, Ukraine. Complex neurological, clinical, laboratory, ultrasound, and neuroimaging examinations were performed to all study patients within 24 hours since the symptoms onset.

Results: MRI/CT-proven thalamic stroke was diagnosed in 34 (10.6%) out of 319 patients, forming a study group. Twenty-two out of 34 patients (average age 61.9 ± 10.2 years) were diagnosed with an acute isolated ischemic thalamic stroke, and 12 patients (average age 59.0 ± 9.6 years) were diagnosed with an acute thalamic hemorrhage.

Conclusions: Specific neurological features of clinical vascular syndromes of acute thalamic stroke in the classical vascular territories were analyzed, compared, and described.

KEYWORDS: thalamus, stroke, thalamic stroke, syndrome, vascular territory

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INTRODUCTION

Notably, stroke is one of the leading causes of mortality and long-term disability worldwide, and the economic costs of treatment and post-stroke care are substantial. Every two seconds, someone in the world will have a stroke. [1-5] According to the World Health Organization, 15 million people suffer stroke worldwide each year. Of these, 5 million die and another 5 million are permanently disabled. [6]. Also, more young people are affected by stroke in low- and middle-income countries. [5, 7] The highest incidence of stroke occurred in east Asia, followed by the eastern European region, whereas the lowest rates were in central Latin America. [7, 8] As populations age, and low-income and middle-income countries go through the epidemiological transition from infectious to non-communicable diseases as the predominant cause of morbidity, together with concomitant increases in modifiable risk factors, it is expected that the burden of stroke will further increase until effective stroke prevention strategies are more widely implemented. [7] Thalamic stroke is not rare, accounting for 11% of posterior circulation infarcts. [9]

The thalamus plays a critical role in supporting cognitive and motor functions, managing our sensitivity to temperature, light, pain, and physical touch. It controls the flow of visual, auditory, and motor information, being also involved in different aspects of learning, memory, speech, language understanding, emotions, motivation, attention, and wakefulness, being in charge of our sense of balance and awareness of our arms and legs. Medical doctors invariably investigate neurological and neuropsychological symptoms of thalamic strokes during an objective examination of the patient, being an integral part of establishing a syndromological, topical, and clinical diagnosis. [10, 11] However, currently, there are not enough published prospective hospital-based cohort studies that report and analyze pathophysiological features and patterns of the occurrence of neurological, neuropsychological, and clinical vascular syndromes of the thalamic stroke in the classical vascular territories, and their topical diagnosis using clinical, neurological, and neuroimaging methods in a prospective hospital-based cohort study. [10-12]

THE AIM

The purpose of this study is to describe and analyze the clinical and neuroimaging features of vascular syndromes of thalamic stroke in the classical vascular territories in a prospective hospital-based cohort study, providing a comprehensive clinical and neuroimaging analysis.

MATERIALS AND METHODS

The methods of the study, inclusion, and exclusion criteria have been reported in detail previously. [11, 12] In brief, only MRI/CT-proven acute thalamic stroke patients age 18 years or older were included in this prospective, hospital-based, cohort study of acute thalamic stroke patients. Institutional ethics board approval was obtained and written informed consent received from all participants or legally authorized representative. All study participants were admitted to the Neurological Center of Oleksandrivska Clinical Hospital, Kyiv, Ukraine, within the first 24 h since the first stroke symptoms occur. All stroke patients were reviewed by at least two board-certified neurologists with training in cerebrovascular diseases. Clinical history, 12-lead electrocardiogram, blood testing, carotid ultrasound, head CT and/or brain MRI were obtained for all study participants. Stroke education programs were provided to all study participants [13, 14].

STATISTICAL ANALYSIS

Parametric and non-parametric statistic methods were applied. A two-sided $p<0.05$ was considered significant for all analyses. All statistical analyses were performed using IBM SPSS Statistics Version 22.

RESULTS

BASIC CHARACTERISTICS OF THE STUDY POPULATION

In total, 319 adult patients with an acute MRI/CT-proven stroke were screened. Among these 319 patients, 204 (63.9%) patients were diagnosed with an acute posterior circulation ischemic stroke, and 115 (36.1%) patients had an intracerebral hemorrhage. Thalamic stroke was diagnosed in 34 (10.6%) out of 319 patients, forming a study group. The breakdown for study group by stroke type was as follows:

- 22 patients (12 men, 10 women aged 50 to 84 years; average age 61.9 ± 10.2 years) were diagnosed with an acute isolated thalamic stroke;
- 12 patients (5 men, 7 women aged 57–75 years; average age 59.0 ± 9.6 years) with a proven diagnosis of an acute thalamic hemorrhage.

Lacunar Stroke was diagnosed in seven (31.8%) out of 22 patients with isolated thalamic stroke (foci diameter ≤ 1.5 cm), being more often determined in the posterolateral adjacent zone (in 5 patients) and less often in the inferolateral classical vascular territory of the thalamus (in 2 patients). *Non-lacunar stroke,* with a diameter of a foci lesion > 1.5 cm, was detected in 15 (68.2%) patients. It arose as a result of occlusion of the thalamic arteries, branches of the posterior cerebral arteries. Ischemic damage to the thalamus in four (18.2%) patients was due to cardioembolism. Eleven (50.8%) patients were diagnosed with atherothrombotic intracranial subtype of ischemic stroke. Nonlacunar thalamic infarcts were mainly localized in the classical thalamic territories (in 11 patients), less often in the border vascular zones (in four patients).

In nine (40.9%) out of 22 patients with isolated thalamic stroke, the lesion was localized in the right thalamus, in 12 (54.5%) – in the left thalamus, and in one (4.6%) case a bilateral lesion of the thalamus was detected. In our study, thalamic strokes were more often localized in the classical vascular territories – in the territory of the inferolateral artery (40.9%) and in the territory of the paramedian artery (27.3%), and less often – in the borderline vascular zones: posterolateral (22.7%) and central (9.1%).

CLINICAL VASCULAR SYNDROMES OF THALAMIC STROKES IN THE CLASSICAL VASCULAR TERRITORIES

In this article, we describe and analyze thalamic strokes in the paramedian and inferolateral classical vascular territories, as there were no study patients with thalamic strokes in the anterior and/or posterior vascular territories.

In our study group, thalamic stroke in the *paramedian vascular territory* (in 6-27.3% of patients) was accompanied by damage to the posteromedian thalamus, including the nucleus of the medial longitudinal fasciculus, the posterior divisions of the dorsomedial and intralaminar nuclei: central, lateral, and parafasicular. Unilateral thalamic stroke in the territory of the paramedian artery causes neuropsychological disturbances, such as a decreased level of consciousness, vertical gaze paresis, cognitive impairment, and personality changes, known as posteromedian thalamic syndrome. Clinically, decreased level of consciousness was detected in all patients: stunning (in 2 patients) was manifested by a restriction of activity and a slowdown in mental reactions; stupor (in 3 patients) was characterized by a deeper depression of consciousness; one patient with bilateral stroke in the territory of the paramedian artery was diagnosed with a deep coma. So, more severe neurological dysfunction and symptoms occurred with a bilateral stroke in the territory of the paramedian artery. It is believed that decreased level of consciousness is a consequence of damage to the posterior parts of the dorsomedial and intralaminar nuclei, as well as an interruption of their connection with the ascending reticular formation and cerebral cortex. [15] Cognitive impairment was manifested with a memory impairment: in one patient with left-sided thalamic lesion in the territory of the paramedian artery, retrograde amnesia (loss of memory for events preceding a stroke) was revealed, and in two patients with right-sided thalamic stroke, anterograde amnesia (loss of memory for events after a stroke) was determined. In one patient with left hemisphere lesion in the territory of the paramedian artery, ideomotor and constructive apraxia were detected. In another case with a lesion in the territory of the paramedian artery of the right thalamus, anosognosia, hemineglect with ignoring the left side of space, and distorted perceptions of reality in the form of hallucinosis were observed. In right-handed patients with left-thalamus lesions, speech disorders were noted. Vertical paresis of the gaze up was detected in three patients with a lesion in the territory of the paramedian artery of the left thalamus. Thalamic stroke

in the territory of the paramedian artery manifested by behavioral syndromes – anomia and dysthymia.

Thalamic stroke in the *inferolateral classical vascular territory* was developed in nine (40.9%) out of 22 study patients. Different degrees of surface sensitivity impairment of the contralateral half of the body were the main neurological symptoms noted in these patients. Hemigipesthesia in six (66.7%) patients was combined with batihypesthesia and contralateral hemiataxia. In seven (77.8%) patients, stroke lesion spread to the adjacent internal capsule, causing sensorimotor syndrome on the opposite to the lesion side. At the same time, in two patients, motor impairment preceded by the sensitivity impairment (sensorimotor stroke). In other five patients, motor and sensitivity impairment coincided in time. In four patients with left-sided lesions in the territory of the inferolateral artery of the thalamus, varying degrees of the emotional sphere dysfunction and various manifestations of dysthymia (longing, apathy, fear) were noted. The most common causes of thalamic stroke in the territory of the inferolateral artery were microangiopathies in patients with arterial hypertension and hypercholesterolemia (five patients), diabetes mellitus (two patients), cardioembolism (two patients).

DISCUSSION

There is a relative paucity of data in the English literature that describe and analyze the clinical and neuroimaging features of vascular syndromes of thalamic stroke in the classical vascular territories in a prospective hospital-based cohort study of Eastern European patients. Based on the vascular supply, neuroanatomical, and neuropathological data, confirmed by neuroimaging techniques, thalamic infarcts have been classically categorized into four thalamic territories, and they are as follows:

1. anterior territory (supplied by the polar/tuberothalamic arteries that arise from the posterior communicating artery);
2. paramedian (supplied by the paramedian arteries that arise from the P1 segment of the posterior cerebral artery);
3. inferolateral (supplied by the thalamogenicular arteries that arise from the P2 segment of the posterior cerebral artery);
4. posterior (supplied by the posterior choroidal arteries that arise from the P2 segment of the posterior cerebral artery).

Our fundings indicates that unilateral stroke in the territory of the paramedian artery manifested with the ***posteromedian thalamic syndrome***. Impaired consciousness and memory, paresis of the gaze up, pronounced neuropsychological disorders, and hallucinosis were the main symptoms of this syndrome. It is believed that the damage to the posterior parts of the dorsomedial and intralaminar nuclei, as well as an interruption of their connection with the ascending reticular formation and the cerebral cortex, causes decreased level of consciousness. [16] Stroke in the territory of the paramedian artery generates excessive impulse to the temporal lobe cortex, which is involved in the perception and processing of visual information, causing the development of hallucinosis. [16] The occurrence of selective upward disturbance of the gaze confirms that thalamic stroke in the territory of the paramedian artery has pathological effect on the supranuclear tracts responsible for vertical control of the gaze without damage to the rostral midbrain. [17]

We found that more severe neurological dysfunction occurred in patients with bilateral stroke in the territory of the paramedian artery. ***Paramedian thalamic stroke syndrome*** with akinetic mutism, amnestic disorders, occurred when the dorsomedial nuclei of the thalamus were affected. In our observation, a thalamic dementia was developed within a year after a stroke in a patient with bilateral stroke in the territory of the paramedian artery. Thalamic dementia occurs when the medial dorsal nuclei of the thalamus were damaged along with the mamillary bodies. [18] Bilateral stroke in the territory of the paramedian artery occurs due to atheromatous or embolic occlusion of the common branch of the thalamosubtalamic artery known as Percheron's artery.

Isolated thalamic stroke in the territory of the inferolateral artery manifested by heterolateral hemihypesthesia and contralateral hemiataxia. Sensitivity impairments were often combined with impaired motor skills due to a damage to the tissue of the internal capsule adjacent to the stroke foci, determining the development of the ***sensorimotor syndrome.***

In patients with extensive inferolateral thalamic stroke, the classic thalamic ***Dejerine–Roussy syndrome*** occurred. This, so-called ***thalamic pain syndrome,*** characterized by moderate contralateral hemiparesis, hemihypesthesia, hemiataxia, hemialgia, paresthesia, as well as dysesthesia (perversion of the perception of sensitive irritations). Dejerine–Roussy syndrome is a neurologic disorder first described by the French neurologist, Jules Joseph Degerin and his student Gustave Roussy, in 1906. Thalamic pain syndrome was revealed in two patients with an extensive ischemic stroke in the territory of the inferolateral artery, being characterized by the following clinical manifestations: moderate transient hemiparesis on the opposite lesion side without side signs of muscle spasticity, severe hemihypesthesia, hemiataxia, pain and paresthesia after a stroke in the distal extremities, often extending to the entire half of the body – hemialgii.

In patients with extensive lesions in the inferolateral vascular territory of the thalamus, a dynamic contracture of the fingers occurred in the opposite to the stroke side, so-called, classic thalamic arm "***main thalamic***" that is described as follows: the forearm is bent and penetrated, the wrist joint is bent, the main phalanges of the fingers are moderately bent, while the middle and end phalanges are fully extended. The fingers of the hand are in continuous motion – ***choreoathetosis***. [11] The described clinical syndrome occurs as a result of damage to the posterior ventral nucleus and ventrolateral thalamic nucleus with the spread of the infarction foci to the inner capsule.

Neurological symptoms in patients with thalamic stroke in the territory of the inferolateral artery can mimic symptoms of ischemic stroke with capsular localization of the lesion (carotid arterial systems). Thalamic pain with all signs of hyperpathia, emotional and autonomic manifestations was pathognomonic for the thalamic stroke. Thalamic syndrome was associated with hemianesthesia, hemiataxia, and hemianopsia even though these symptoms are not pathognomonic for thalamic stroke. In addition to the sensitivity impairment, hemialgia, paresthesia, psychosensory impairment (hallucinosis), choreoathetosis, dystonic manifestations with a tendency to contractures of a dynamic type, and emotional central paresis of facial muscles were noticed. In all patients with thalamic stroke in the territory of the inferolateral artery, varying degrees of cognitive status impairments were present. The most significant cognitive impairment was observed in patients with Degerin-Roussy syndrome.

CONCLUSIONS

Summarizing, we would like to highlight that the unilateral thalamic stroke in the territory of the paramedian artery typically manifested by the posteromedian syndrome (decreased level of consciousness, paresis of the gaze up, cognitive impairment); more severe symptoms were observed in a case of a bilateral stroke (paramedian thalamic infarction syndrome). Severe emotional dysfunction, such as syndromes of anomie and dysthymia, were typical in patients with thalamic stroke in the territory of the paramedian artery.

Isolated thalamic stroke in the territory of the inferolateral artery manifested by a syndrome of heterolateral hemianesthesia, hemataxia, combined with impaired motor skills, the presence of a pain, autonomic disorders with signs of hyperpathia, cognitive deficit; a specific sign was the development of thalamic syndrome Dejerine-Roussy; with an extensive stroke lesions, a dynamic contracture of the fingers of the hand occurred – the classic "thalamic arm" ("main thalamic"); the spread of a stroke to the adjacent inner capsule caused the development of sensorimotor syndrome. The understanding of the vascular syndromes of the thalamic strokes was and remains an extremely important component of the supervision of a patient with thalamic stroke, and is an integral part of establishing a topical and clinical diagnosis.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

PREVENTION OF PREECLAMPSIA IN WOMEN WITH MULTIPLE PREGNANCY AFTER ASSISTED REPRODUCTION

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ABSTRACT

The aim: To reduce the frequency and severity of preeclampsia, to improve obstetrical and perinatal outcomes in women with multiple pregnancy after assisted reproduction by the development and implementation of the preventive algorithm with biochemical markers of endothelial dysfunction prospective analysis.

Materials and methods: Clinical and laboratory prospective analysis of 54 cases of twins in women, treated from infertility with assisted reproductive technologies (ART), using the method of intracytoplasmic sperm injection (ICSI) and frozen embryos transfer, have been made. It was proven, that women with multiple pregnancy are always in a high risk group of placental dysfunction (PD) and preeclampsia (PE). Depending on the treatment algorithm and preventive measures, 2 groups of patients were formed. Group I included 29 pregnant women with twins, managed in accordance with developed recommendations. We didn't find evidence-based European guidelines, that would recommend routine prescription of progesterone to improve chorion invasion and further placentation in such group of patients, but in order to prevent endothelial dysfunction and to decrease the incidence and severity of preeclampsia, placental abnormalities and intrauterine growth restriction (IUGR), we proposed the following algorithm: – micronized progesterone 200 mg vaginally (PV), as soon as pregnancy was diagnosed by positive hCG-test, till 16 weeks of pregnancy, angioprotector diosmin 600 mg once daily orally (PO), 2 courses: from 8 till 12 and from 16 till 20 weeks of gestation, antiaggregant – acetylsalicylic acid 150 mg from 12 till 36 weeks of gestation. Group II included 25 pregnant women with twins after the same ART procedures, who have not received above mentioned treatment. Plasma concentrations of PlGF, sFlt-1 and the ratio of sFlt-1/PlGF in the second trimester were investigated in both groups of women in order to assess the effectiveness of proposed preventive measures.

Results: Usage of preventive algorithm has shown the reduction of PE incidences in 26%, PD in 28.1%, IUGR in 35%, prematurity by 23% and fetal distress in 18%, that led to improvement of obstetrical and perinatal outcomes in I group of women with multiple pregnancies after ART-treated infertility, compared with group II ($p<0.05$). The evaluation of PlGF, sFlt-1 plasma concentrations and the ratio of sFlt-1/PlGF in the second trimester of pregnancy reflected the effectiveness of our method in women with twins after ART. The level of PlGF in the study group was higher (186.5 ± 12 vs 154.2 ± 10.7; $p<0.05$), and the level of sFlt-1 was lower (1523.1 ± 40.3 vs 1835.3 ± 33.6; $p<0.05$). Results of sFlt-1/PlGF ratio analysis in the I group also showed effectiveness of the method proposed (20.3 ± 3.1 vs 28.1 ± 2.2; $p<0.05$).

Conclusions: The observed results suggest, that pregnant women with twins after ART-treated infertility are in a high-risk group of PE, PD and IUGR of one or both fetuses. Implementation of the proposed preventive algorithm allows to reduce the incidence of PE, obstetrical and perinatal complications in this group of patients, and can be widely used in clinical practice. Evaluation and prospective assessment of biochemical markers, such as PlGF, sFlt-1 and sFlt-1/PlGF ratio, in the second trimester of pregnancy in the target groups may likely predict the development of PE and its severity.

KEY WORDS: preeclampsia, placental disfunction, multiple pregnancy, twins, acetylsalicylic acid, prevention, diosmin, placental growth factor (PlGF), soluble form of vascular endothelial growth factor (sFlt-1), PlGF / sFlt-1 ratio, assisted reproduction, endothelial dysfunction

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INTRODUCTION

Annually, about 76,000 women and 500,000 children die due to preeclampsia (PE). Among women with complicated pregnancy (preeclampsia, gestational diabetes, gestational hypertension, intrauterine growth restriction (IUGR), placental detachment, etc.) there is 3.7-fold increase of hypertension, 2.2-fold increase of ischemic heart diseases, 2.7-fold increase of cardio-vascular diseases in later life [1, 2].

According to ACOG, multiple pregnancy adue to assisted reproductive technologies is associated with an increased risk of preeclampsia (OR 2.7). A study of Opdahl S. et al [3] in the 2015 not only confirmed the high incidence of gestational hypertension, but also found that the incidence is higher in case after ART. Hypertensive disorders after ART were observed in 5.9% of singletone pregnancies and in 12.6% of multiple pregnancies. In all cases, regardless of ART technology in singletone pregnancies the risk was the same, but it was the highest when using frozen embryos (risk 7.0%, risk difference 1.8%, CI 1.2-2.8). In twin pregnancies, the risk was also the highest when using frozen embryos (risk 19.6%, risk difference 5.1%, CI 3.7-7.1). In 2017 Storgaard M. et al [4] found that during pregnancy using the donor egg comparison with conventional pregnancies by ART, the risk of preeclampsia was the highest, with odds correction coefficient OR, and was 2.11 (CI 1,42-3,15) in single pregnancies and OR 3,31 (CI 1,61-6,80) with twins.

Thus, according to statistics, the incidence of severe preeclampsia in multiple pregnancies is 3-4 times higher

than in singletone pregnancies [5].

Numerous recent works are dedicated to search preeclampsia predictive markers. It should be noted at the same time that an activive research is made in order to find a universal test for determining the risk of complications during pregnancy, with the emphasis on molecular genetic markers. It has been repeatedly proven that angiogenesis is a key process in the formation of the placental vascular system. Angiogenic factors and their receptors are important regulators of placental vascular system [6, 7].

The complete formation of uterine arteries is regulated by proangiogenic (promoting the growth of the endothelium) and anti-angiogenic biological substances. The placental growth factor PlGF (placental growth factor) is mainly produced by trophoblast. During the pregnancy trophoblast and VEGF (vascular endothelial growth factor) belong to the first group. Their antagonist is soluble tyrosine kinase sFlt-1 (soluble fms-like tyrosinekinase). During the physiological pregnancy, there is a normal balance between these indicators – a certain amount of PlGF is synthesized, which binds to Flt-1 receptors located on the surface of the endothelial cells and promotes vasodilation of the uterine arteries. Excess of PlGF «utilized» by action of sFlt-1. However, the process may be altered, and for some reason (not determined completely) promoting increased synthesis of sFlt-1 at normal amount of synthesized PlGF. This imbalance leads to increased binding of the placental growth factor by tyrosine kinase. Due to the lack of free PlGF, Flt-1 receptors do not receive the required amount of proangiogenic factor that causes vasoconstriction, the uterine arteries remain constricted, and blood flow is accelerated. This situation triggers a cascade of further pathophysiological changes that ultimately leads to serious health disorders in a pregnant woman organism and problems with fetal growth [8,9,10].

According to published reports, antiangiogenic factors predominate in pregnant with twins. Interesting that the same group of pregnant women, but after ART presented with even more pronounced anti-angiogenesis[6,11,12]. The concentration of circulating biomarkers of sFlt-1 and PIGF changes in women with PE even before its development and correlates with severity and early onset. In cases where PE subsequently developed, low levels of PIGF in the I trimester was observed and the level of sFlt-1 can start to increse 5 weeks before its onset. Thus, in the II trimester, the ratio of sFlt-1 and PIGF may serve as an informative marker of PE. Analysis of data from a multicenter prospective cohort study of pregnancies (n = 772) showed that in women with sigletone pregnancies the concentration of circulating sFltl and PIGF is lower than in multiple pregnancy [13].

The level of sFlt-1 in case of physiological pregnancy begins to increase 5 weeks before the possible occurrence of the first clinical manifestations of PE, and further increases. There is a direct correlation between the level of sFlt-1, severity PE and the development of hypertension and proteinuria [9]. A decrease in PIGF level or increase of sFLT-1 / PIGF ratio during pregnancy is considered as a prognostic indicator of PE development [9, 14]. In early and late forms of PE, the level of sFlt-1 in the mother's blood changes. Several studies have shown a 43-fold increase of sFlt-1 concentrations in case of early forms of PE and a 3-fold increase in case of late as compared to the concentration of sFlt-1 on the background of physiological pregnancy. It is believed that the increase of sFlt-1 level in the II trimester reliably corresponds to development of PE and is the most accurate marker of the development of its early form [15].

S. Verlohren et al. confirmed the high diagnostic value of Flt-1 / PIGF ratio in the period from 20 to 34 weeksof pregnancy as a marker of PE with a sensitivity of 95% and specificity of 94% [16].

All of the above is a clear justification for the relevance of the chosen research direction.

THE AIM

To reduce the frequency and severity of preeclampsia, to improve perinatal and obstetrical delivery outcomes in pregnant women with multiple pregnancy after in vitro fertilization due to improvement and implementation of the preventive measure algorithm and studying biochemical markers of endothelial dysfunction.

MATERIALS AND METHODS

According to this goal were conducted a prospective analysis of 54 twin pregnancies for the period from 2017 to 2019. Those pregnancies were achieved through assisted reproductive technology (ART), using the method of intracytoplasmic sperm injection (ICSI) and frozen embryos transfer. The usage of frozen embryos always leads to an increased risk of placental dysfunction (PD) and preeclampsia. Depending on the algorithm of treatment and preventive measures of complications, 2 groups were formed. Group I included 29 women pregnant with twins. They were offered the following algorithm: – micronized progesterone 200 mg PV from the first day of pregnancy to 16 weeks of pregnancy; – angioprotector diosmin 600 mg once a day, per os, 2 courses: 8 to 12 and 16 to 20 weeks of gestation; antiaggregant – acetylsalicylic acid 150 mg from 12 to 36 weeks of pregnancy. Group II included 25 pregnant women with twins who didn't get the listed above treatment.

We propose the following algorithm of treatment and prophylactic to reduce the incidence of preeclampsia, obstetric and perinatal complications in pregnant women with multiple pregnancy after ART:

- hormone therapy: micronized progesterone 200 mg per vaginum from the first day of pregnancy to 16 weeks;
- angioprotective therapy: diosmin 600 mg per os 2 courses 8 to 12 weeks, 16 to 20 weeks;
- antiagregant therapy: acetylsalicylic acid 150 mg for 12 to 36 weeks.

PIGF, sFlt-1, and sFlt-1 / PIGF ratios were studied in both trimesters in both groups. The effectiveness of the proposed treatment-and-prophylaxis algorithm was evaluated by perinatal and obstetric outcomes, plasma PIGF, sFlt-1, and sFlt-1 / PIGF ratios in the second trimester. Microsoft Excel XP and Statistica 6.0 Windows, methods of descriptive

Table I. Peculiarities of pregnancy

Complications	I group (n=29)	II group (n=22)
Preeclampsia:	10,4% (3)*	36,4% (8)
moderate	6,9% (2)	18,2% (4)
severe	3,5% (1)	18,2% (4)
Placental dysfunction:	17,3% (5)*	45,5% (10)
IUGR (degree):	10,4% (3)	45,5% (10)
I	6,9% (2)	13,6% (3)
II	3,5% (1)	22,8% (5)
III	-	9,1% (2)
Fetal distress	13,8% (4)	31,8% (7)
Preterm labor	17,3 % (5)	36,4% (8)
Before 34 weeks of gestation	6,9% (2)	22,8% (5)
After 34 weeks of gestation	10,4% (3)	13,6% (3)
PANSP	-	9,1% (2)*

Note: *p <0.05 compared to group II indicators

Table II. Features of newborns condition

Complications	I group (n=58)	II group(n=44)
Hypoxic-ischemic CNS injury	6,9% (4) *	15,9% (7)
Disorders of adaptation	17,2% (10) *	38,6% (17)
Transfer of neonates to 2ndstage of treatment	6,9% (4) *	25% (11)

Note: *p <0.05 compared to group II indicators

Table III. Biochemical markers of preeclampsia in the second trimester

Value	I group (n=29)	II group (n=22)
PlGF	186,5 ± 12,6	154,2 ± 10,7
sFlt-1	1523,1 ± 40,3	1835,3 ± 33,6
sFlt-1/PlGF	20,3 ± 3,1*	28,1 ± 2,2

Note: *p<0,05 compared to Group II values

statistics and correlation analysis were used for statistical processing.

RESULTS

Analysis of the clinical course and termination of pregnancy showed significantly better results in group I (p <0.05 compared to group II indicators) (Table I).

The incidence of preeclampsia in group I was only in 10.4% cases (3) versus in group II 36.4% cases (8) (group I had 1case (3.5%) of severe preeclampsia and 2 (6.9%) cases of moderate preeclampsia; in group II moderate and severe preeclampsia were observed in 18.2% (4) and 18.2% cases (4) respectively). Placental dysfunction was diagnosed in 17.3% (5) of group I cases and in 45.5% (10) of group II. Intrauterine growth restriction was less common in Group I – 10.4% (3) (I degree 6.9% (2), II degree in 3.5% (1), no cases of III degree in comparison with 45.5 % cases (10) in Group II (I degree 13.6% (3), II degree 22.8% (5), III degree 9.1% (2). Fetal distress was diagnosed in 4 pregnant women (13.8%) of group I, and in 7 women of group II (31.8%). We recorded 5 (17,3%) preterm labor (before 34 weeks of gestation – 2(6,9%); after 34 weeks of gestation – 3(10,4%) in the group where the proposed prevention regimen was applied. In the group where the conventional regimen was applied there were 8 cases (36.4%) of preterm labor(before 34 weeks of gestation – 5 (22.8%); and 3 cases (13.6%) after 34 weeks of gestation. Placental abruption was diagnosed in 9.1% (2) among group II, and in group I no such cases were recorded. The peculiarityof newborns' status are shown in table II.

Hypoxic-ischemic injuries occurred in 4 (6.9%) children of group I and in 7 (15.9%) children of group II. Disorders of adaptation were observed significantly less in the group using the proposed scheme prevention – 10 (17.2%) cases, compared to group II – 17 (38.6%) cases. Transfer to the second stage of treatment required 4 (6.9%) neonates in group I and 11 (25%) neonates in group II.

DISCUSSION

An investigation of plasma concentrations of PlGF, sFlt-1, and the sFlt-1 / PlGF ratio in the second trimester of pregnancy confirmed the effectiveness of treatment and preventive measures (Table III), namely: the level of PlGF in the study group was higher (186.5 ± 12.6 opposite to 154.2 ± 10.7,

p<0,05) and the level of sFlt-1 lower (1523.1 ± 40.3 vs. 1835.3 ± 33.6,p<0,05).The results of the sFlt-1 / PlGF ratio in the study group also indicated a better effect of the proposed prophylaxis method (20.3 ± 3.1 vs. 28.1 ± 2.2, p<0,05).

Usage of our advanced preventive measures algorithm can reduce the incidence of preeclampsia by 26%, placental dysfunction by 28.1%, preterm labors by 23%, fetal distress by 18%, fetal growth retardation by 35%, which together improves obstetric and perinatal outcomesin case of multiple pregnancy after ART.

CONCLUSIONS

Thus, the results of the study indicate that pregnant women with twins achieved through ART are at high risk of preeclampsia, placental dysfunction, and fetal growth retardation. Modern scientific methods of prevention of obstetric and perinatal complications are needed in order to solve this problem. The proposed algorithm of modern diagnostic and preventive measures allowes to significantly reduce the incidence of preeclampsia, obstetrical and perinatal complications in this group of pregnant women and can be used in practical public health care. Determination of the biochemical markers, such as PlGF, sFlt-1 and sFlt-1 / PIGF ratio in the second trimester may be likely to predict the development of preeclampsia and its severity.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

ULTRASTRUCTURAL FEATURES OF HERPESVIRUS-INDUCED AORTIC INTIMA DAMAGE IN MICE FED THE HIGH-FAT DIET

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ABSTRACT

The aim: To study the effect of a high-fat diet (HFD) on the structural changes in the aortic intima in intact and HSV-1-infected mice using Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM).

Materials and methods: In experiments Balb/c mice were infected with the HVS-1 and fed high-fat diet and 12 weeks later aortic ultrastructure was examined by SEM and TEM methods. The animals were subdivided into four experimental groups: 1st group – HSV-1-infected animals; 2nd – animals consuming high-fat diet (HFD); 3rd – infected animals that were subsequently consuming a high-fat diet (HSV / HFD); 4th – animals consuming a high-fat diet that were subsequently infected with HSV-1 (HFD / HSV) (n = 6); and control group – intact animals.

Results: HVS-1 impaired ultrastructural changes in aorta greater than high-fat diet and HVS-1 alone (higher density of lipid inclusions in the subendothelial space, necrosis of endothelial cells), and infection of mice after high-fat diet ended 100% mortality. The formation of atheroma in the aortic wall during HFD was not detected, but the initiative manifestations of atherogenesis have been identified and restricted in the aortic intima. These structural changes included lipid inclusions in the subendothelial space, cell damage and destruction, which lead to an increase cellular detritus in the 3rd (HSV / HFD) group.

Conclusions: HSV infection potentiates the accumulation of lipid inclusions in the aortic intima during a HFD, facilitates infection and contributes to the development of acute infection.

KEY WORDS: aorta, high-fat diet, HVS-1, atherogenesis, electron microscopy

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INTRODUCTION

Hyperlipidemia and hypercholesterolemia are major biochemical risk factors for atherogenesis, which ultimately contribute to atherothrombosis with severe consequences. Currently, there is a small number of research articles dedicated to the structural changes in the aorta caused by hyperlipidemia and atherosclerosis as well as HSV-induced atherosclerosis [1]. Even less information is available on morphological studies of the aorta affected by a viral infection associated with atherogenesis. HSV and CMV were detected in 40% of the examined autopsy samples of the aorta and coronary vessels [2]. In experiments, the effect of HSV-infection on atherosclerosis has been studied fragmentarily, but nevertheless they demonstrate the formation of atheroma in mice with murine gammaherpesvirus-68 (MHV-68) and its progression within 24 weeks compared to the control group [3]. Murine CMV (MCMV) increases the incidence rate of atherothrombosis in apoE-knockout mice by 84%, whereas Chlamydia pneumoniae (Cpn) are not associated with an increased rate of the disease [4]. Morphological signs of atherosclerosis are described in the aortic sinus in apoE-knockout mice [5].The fact that HSV-1-induced atherothrombosis does not reach the values of atheroma is linked to the development of systemic infection, even though HSV-infection promotes thrombosis and increases the risk of atherothrombosis [6].

It is currently widely recognized that any viral infection that damages endothelial cells of blood vessels can be reliably associated with the risk of vascular disease [7]. Biochemical analysis revealed that content of free and esterified cholesterol was higherin the aortic tissue specimens of CMV-infected animals with normocholesterolemia than in the models of non-infected animals. Thus, according to Fabricant C., herpesvirus infection contributed to the accumulation of lipids in the aorta [8], and previous immunization prevented atherosclerotic and fibroproliferative changes in the aortic wall [9] due to decreased activity of cytoplasmic cholesterol esterase in animals [10]. The findings were consistent with accumulation of saturated cholesterol and triacylglycerol due to impaired hydrolysis observed in the HSV-1-infected smooth muscle cells in humans [11]. The scholars describe the smooth muscle cells involved in the active synthesis of extracellular matrix of the connective tissue, such as glycosaminoglycans, collagen and elastin [12]. It is believed that the described cells originate from the media, but penetrate into the intima through the damaged inner elastic membrane of the aorta, and these altering changes are an early manifestation of atherogenesis in the intima of hypercholesterolemic animals [13].

Our hypothesis is that previous viral infection induces structural and functional changes in the tunica intima of

blood vessels that may promote the accumulation of lipid inclusions in the aortic intima in animals consuming a high-fat and high-cholesterol diet.

THE AIM

To study the effect of a high-fat diet (HFD) on the structural changes in the aortic intima in intact and HSV-1-infected mice using Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM).

MATERIALS AND METHODS

Balb/c mice were used in the experiments considered here. The study included 5 groups of the murine aortic tissue samples:
Control group (n = 3) – intact animals;
Group 1 – HSV-1-infected animals (n = 4);
Group 2 – animals consuming high-fat diet (HFD) (n = 6);
Group 3 – HSV-1-infected animals that were subsequently consuming a high-fat diet (HSV / HFD) (n = 6);
Group 4 – animals consuming a high-fat diet that were subsequently infected with HSV-1 (HFD / HSV) (n = 6).
The animals in the 1st group were infected with HSV-1 in L.V. Gromashevsky Institute of Epidemiology and Infectious Diseases, NAMS of Ukraine (Head of the Laboratory of Experimental Chemotherapy of Viral Infections, MD, Prof. S.L. Rybalko).

In a 12-week experiment, the animals in the 2nd group received the food with cholesterol, animal and vegetable fats. 100 g of food contained 30-45 g of heat-treated animal fats (pork fat / lard, butter); 15-20 g of heat-treated vegetable fats (margarine); 1.5-2 g of cholesterol; 1 g of calcium carbonate ($CaCO_3$); 10 mg (in terms of daily intake for animals – 10 mg / kg) of Mercazolyl (1-methyl 2-mercaptoimidazole with potato starch, Calcium stearate, refined sugar, talcum), 32-52 g of standard granulated animal feed.

The animals in the 3rd group were infected with HSV-1 and then received a high-fat diet as above.

The animals in the 4th group received a high-fat diet and then were infected with HSV-1 as above.

After 12 weeks of the experiment, all animals were withdrawn from it by decapitation. After decapitation, SEM and TEM were used in the study.

SEM was used to study: 1) changes in the intima of blood vessels (surface, integrity of the endothelial layer, erythrocytes and leukocytes adhesion, thrombosis); 2) cellular and non-cellular entities that affect the condition and structural integrity of the intima. SEM is an effective method that facilitates the detection of any changes in the surface of the inner layer of the aorta and makes it possible to reveal any structural lesions on a larger area compared to TEM [11]. TEM was used to study dystrophic changes involving 1) intimal endothelial cells (necrosis, vacuolisation), 2) subendothelial zone (collagen fibers, lipid inclusions); 3) smooth muscle cells.

The aorta was selected for SEM and TEM. For SEM, the aortic tissue fragments of 1-2 mm in size were fixed in 10%phosphate buffered (PBS) formalin solution (pH = 7.4). After fixation, the specimens were washed with PBS, dehydrated in ethanol, dried under a critical point of CO2. The dried samples were coated with gold in a thickness of 15 nm using the Gatan 682 PECS device. The specimens were examined under an electron microscope Tescan Mira 3 LMU (Czech Republic) in scan mode. For TEM, the aortic tissue fragments of 0.3-0.5 mm in size were fixed in 2.5% glutaraldehyde solution on PBS with further fixation in 1% buffered osmium tetroxide solution (OsO4); dehydration in ethanol (70%, 80%, 90%, 100%) and acetone; impregnated and embedded in a mixture of epoxy resins (Epon 812, Araldite 502). The Reichert ultramicrotome was used to cut the epoxy blocks to produce ultra-thin slices of material for study. The ultra-thin sections were contrasted in 2% solution of uranyl acetate and lead citrate. The material was explored and photographed under an electron microscope Tescan Mira 3 LMU (Czech Republic) in illumination mode. TEM was used to investigate the ultrastructural changes in endothelial cells, extracellular matrix, and smooth muscle cells. The use of SEM ensured the investigation of the aortic intimal surface as well as intimal lesions within the aorta.

The heart of HSV-1-infected mice was selected to identify the virus in vitro. Homogenates were obtained from the specimens and added to a cultured Vero cell line grown in sterile plates («Nunc»). Composition of the culture medium: 88% of RPMI 1640 («Sigma»), 12% of inactivated by heating serum of the embryo of the cow («PanEco» RF) and antibiotics. Cultivation conditions: 37°C, 5% CO2. Detection of the virus, reproduction in Vero cells, and passages were performed on the basis of cytopathic effect registration (CPE). Cytological signs of CPE – syncytia. The CPE was recorded for 6-7 days.

Statistical processing of the results was carried out using the Origin Lab program, version 8.0. The normal distribution of the results was estimated by the Kolmogorov-Smirnov criterion. The intergroup discrepancies between the groups of samples were evaluated using nonparametric Kruskal-Wallis test. The difference was estimated to be significant at $p < 0.05$.

RESULTS

TEM and SEM were performed to detect aortic changes in the control group, 1st, 2nd and 3rd groups (HSV, HFD and HSV / HFD). The specimens in the 4th (HFD / HSV) group were not analyzed because the group had an absolute (100% n = 6) mortality in the first 3-5 days after infection. However, the heart of each infected animal was selected for virological examination.

Low-magnification SEM images (×1100 – ×1500) showed a longitudinal waviness of the aortic lumen in intact animals, which is physiological. In the 1st (HSV), 2nd (HFD) and 3rd (HSV / HFD) groups, the surface had the same physiological waviness that correlates with the presence of the inner elastic membrane in the aorta and indicates its preservation.

High-magnification SEM images (×8000 – ×13000) revealed an irregular surface with abundant cellular

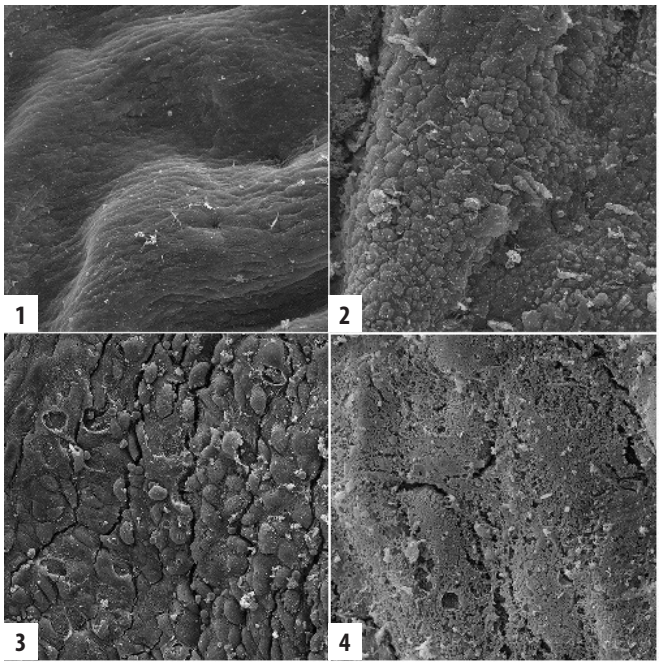

Fig. 1. Scanning electron microscope (SEM) images of the murine aortic wall. Intact and smooth surface of the aortic intima in control; swelling and protrusion of the surface of the aortic intimal cells in the 1st (HSV) and 2nd (HFD) groups; disrupted and coagulated intimal cells in the 3rd (HSV / HFD) group. Note: 1) control (× 10500); 2) 2nd (HFD) group (× 12700); 3) 1st (HSV) group (× 9210); 4) 3rd (HSV / HFD) group (× 8250).

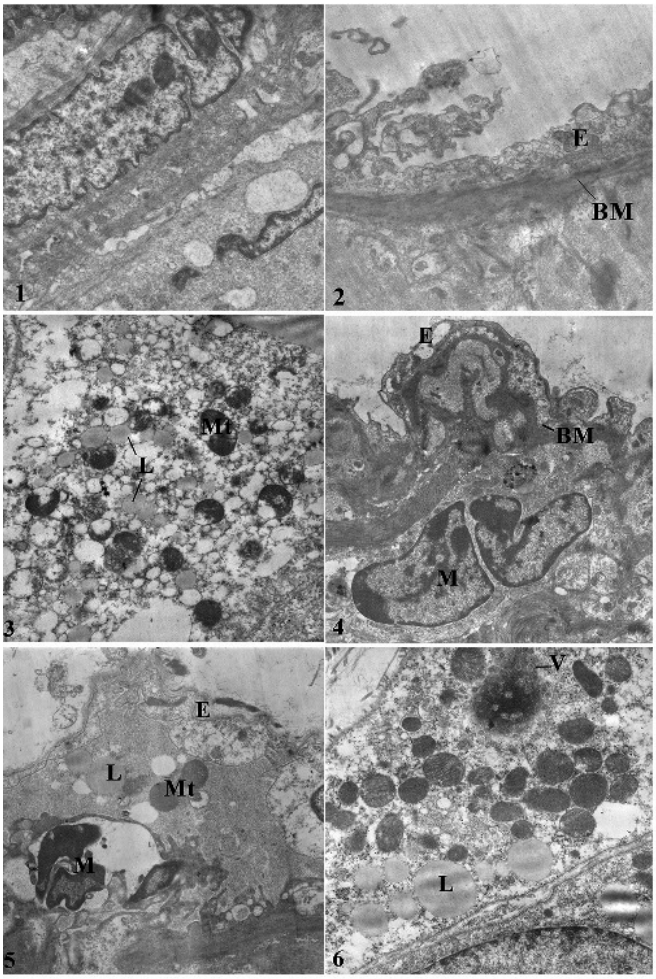

Fig. 2. Transmission electron microscope (TEM) images of endothelial cells and smooth muscle cells in the murine aorta. 1) control group: intact smooth muscle cell (TEM, × 15600); 2) 2nd (HFD) group: destruction and desquamation of endothelial cells in the aortic lumen (× 15600); 3) 2nd (HFD) group: phagocytosis of lipid inclusions by a macrophage («foam cell») (× 15600); 4) 1st group (HSV): altered contour of the inner layer, vesicles in endothelial cells, monocyte with phagosomes in the subendothelial region (× 15600); 5) 3rd (HSV / HFD) group: destruction and desquamation of endothelium, macrophages in the subendothelial space (× 15600); 6) 3rd (HSV / HFD) group: lipid inclusions and virions in the cytoplasm of the cells in the muscle membrane (× 15600). E – endothelial layer; M – monocyte / macrophages; BL – basal lamina; V – virions; L – lipid inclusions; Mt – mitochondria.

protrusions in the 2nd (HFD) group (Fig. 1). This is due to edema and elevation of endothelial cells, their nuclear zone, swelling of cytoplasm above the basal membrane. The accumulation of extracellular matrix derivates and necrotic cells in the subendothelial space is not excluded.

In the 1st (HSV) and 3rd (HSV / HFD) groups, SEM showed adhesion of leukocytes on the surface of the aortic intima in all areas. Additionally, TEM registered leukocytes in the subendothelial zone, which can be explained by their penetration through the endothelial layer. There were no gaps in the endothelium and platelet adhesion detected in the 1st (HSV) and 2nd (HFD) groups, whereas in the 3rd (HSV / HFD) group, we recorded necrotized endothelial cells, signs of coagulation and loss of intimal integrity.

The surface of the intima had additional signs of destructive changes in the form of fibrous structures that differed from the intact aorta. Those were damaged endothelial cells and most likely coagulated proteins. Such changes were more prominent in the 3rd (HSV / HFD) than in the 1st (HSV) group (Fig. 1).

On the surface of the intima in the 2nd (HFD) group, erythrocyte adhesion and coagulation products were observed, but leukocytes were found less frequently. All groups had focal zones of disorganization and loss of endothelial lining (disorganization), which distinguished experimental samples from intact aortic specimens. According to SEM, erythrocyte adhesion and endothelial cell edema, seen as focal protrusions on the surface, were denser in the 2nd (HFD) than in the 1st (HSV) and 3rd (HSV / HFD) groups (unfortunately, quantitative analysis is impossible). Damage and loss of endothelial cells in the intima were more pronounced in the 1st (HSV) and 3rd (HSV / HFD) groups than in the 2nd (HFD) group due to the presence of a viral infection, which can explain more severe necrotic and lytic changes in endothelial cells (with the formation of focal regions, where endothelium is irregular or absent).

TEM was conducted to evaluate the following structural elements of the aortic wall: 1) endothelial layer; 2) subendothelial zone; 3) muscle membrane. In the control group, the aortic intima was unchanged: the nuclei of endothelial cells had a different distribution of chromatin, cytoplasmic

Table I. Results of quantitative measurements in comparison groups (Me [Q1-Q3])

Group	Infectious titer, lg CPE 50	Diameter of lipid inclusions, micron	Diameter of atypical vesicles in endothelial cells, micron
Control	-	-	-
Group 1 (HFD)	-	0,48[0,30-0,68]	0,38[0,23-0,47]
Group 2 (HSV)	1[1-2]	-	0,29[0,19-0,38]
Group 3 (HSV/HFD)	2[1-3]	0,66[0,46-0,98]*	0,68[0,43-0,67]*#
Group 4 (HFD/HSV)	3,5[2,75-4]#	NA	NA

Note: * - significant difference with the 2nd (HFD) group;
- significant difference with the 1st (HSV) group; NA - not analyzed

pinocytotic vesicles; in smooth muscle cells, intact organelles (ribosomes, vesicles, mitochondria, endoplasmic reticulum) were found (Fig. 2).

In the 2nd (HFD) group, SEM showed organelle reduction, edema and destruction of mitochondria, deformation of the nuclei in endothelial cells. Endothelium atypically protrudes into the lumen of vessel and separates the derivates of cell destruction into the aortic lumen, which explains the appearance of the altered intimal contour. In some cells pinocytotic vesicles were found (the evidence of partial preservation of transendothelial transport). In the endothelium cytoplasm, there were detected atypical vesicles, most likely formed as a result of swelling of membrane organelles, in particular mitochondria. Their diameter was evaluated morphometrically (Table 1).

The inner elastic membrane of the aorta was structurally preserved. In the subendothelial zone of the aorta, lipid inclusions were detected. The diameter of lipid inclusions in macrophages was in the range of 150-230 nm, and in the extracellular matrix it even reached 4 microns (Table I).

Lipid deposition contributed to the transformation of cholesterol-engorged macrophages into so-called «foam cells», destruction of individual cells and the appearance of cell free zones. Some cells were in a state of total destruction.

The morphological organization and the integrity of the murine aortic muscle membrane were preserved in the 2nd (HFD) group, even though some changes in smooth muscle cells were found: 1) edema and destruction of cristae of mitochondria, 2) appearance of atypical vesicles in the cytoplasm, and 3) reduction of organelles and myofibrils. In some cells, accumulation of secondary lysosomes was observed. These changes indicate a secondary metabolic injury.

In endothelial cells of the 1st (HSV) group, the following changes were recorded: 1) cytoplasmic edema; 2) lysis of organelles; 3) atypical vesicles, which can be considered as a consequence of lytic changes and fusion of membrane organelles. In this case, the integrity of the endothelial layer was preserved. Under the endothelium, the basal membrane, internal elastic membrane, and subendothelial layer of the inner aortic membrane were observed. In subendothelial zone, the following changes were revealed: 1) macrophages; 2) diapedesis of some blood corpuscles, 3) decrease in the density of collagen fibers. The revealed changes indicate a decrease in: 1) the barrier function of endothelial cells, 2) the infiltration of leukocytes; 3) the reorganization of paravasal intercellular space, which is a manifestation of the inflammatory reaction and is a qualitative morphological distinction from the 2nd (HFD) group. Under the elastic membrane, smooth muscle cells of the aortic media were found without any significant structural changes.

The 3rd (HSV / HFD) group had progressive structural changes, as described in the 1st (HFD) group. Changes in the endothelium in the form of: 1) edema and destruction of organelles, 2) the appearance of atypical vesicles of greater diameter; 3) necrosis of cells with desquamation and endothelial damage. During SEM these changes were seen as: 1) sites of destruction, coagulation; 2) loss of the endothelial cells (Table 1). The increase in the density and diameter of atypical vesicles by 57.8% ($P = 0.05$) indicates the progression of dystrophic changes in the aortic endothelial cells in the 3rd (HSV / HFD) group compared to the 2nd (HFD) group. Accumulation of virions (diameter 120-150 nm) in injured cell was detected in the one sample.

In the subendothelial layer, the following changes were also revealed: 1) accumulation of lipid inclusions, 2) destructive changes, 3) reorganization of the extracellular matrix and infiltration of macrophages. The diameter of lipid inclusions was 37.5% higher ($P < 0.05$) compared to the 2nd (HFD) group. The density of macrophages with «foamy» cytoplasm also increased, and the reorganization of collagen fibers can be explained by phagocytic activity of macrophages and their participation in remodeling of extracellular matrix. This indicates a higher degree of structural damage to the aorta in the 3rd (HSV / HFD) group compared to the 2nd (HFD) group, that is HSV1 potentiates their development.

The morphological integrity of the muscle membrane was preserved. But, whereas in the 2nd (HFD) group only atypical vesicles were detected in smooth muscle cells, in the 3rd (HSV / HFD) group, perinuclear edema of the nucleus was additionally revealed. This indicates that the presence of HSV infection potentiates the development of ultrastructural changes in the aortic media. Changes in smooth muscle cells are nonspecific and most likely related to metabolic disorders caused by a 12-week consumption of a high-fat diet in the 2nd (HFD) group.

TEM demonstrated that injured aortic intima had insufficient number of ultrastructural markers to identify and verify the damage factor. Lipid inclusions occurred

in the 2nd (HFD) and 3rd (HSV / HFD) groups, and signs of viral damage to endothelial cells (lamellar cells, single virions) were detected only in one samples from the 3rd (HSV / HFD) group.

In vitro studies in all cardiac homogenates confirmed the presence of HSV-1, and the level of reproduction was estimated according to CPE (Table 1). Although there was no significant difference between the 1st (HSV) and the 3rd (HSV / HFD) groups, fatal cases of another model of the 4th group (HFD / HSV) caused significantly higher CPE in the hearts. It is clear that the term of infection period is different, but the absolute lethality of mice that received a high-fat diet for 12 weeks and then were infected with HSV-1, gives reason to think about a certain direct and inverse relationship in the study.

DISCUSSION

Changes in endothelial cells and extracellular matrix were more pronounced in the 3rd (HSV / HFD) group compared to the 2nd (HFD) group. The most striking changes in the aortic intima were observed as the accumulation of lipid inclusions and macrophages in the subendothelial space. The revealed changes in the subendothelial space of the aorta can be explained by: 1) migration of monocytes / macrophages from blood or regional phagocytes for the elimination of lipid inclusions in the 2nd (HFD) group; 2) adhesion and migration of leukocytes to the vascular wall for aggressive response to HSV-1 in the 1st (HSV) group; 3) lipid deposition of cellulardebris in the extracellular matrix and macrophages (lysosomal deposits) promoted by a structurally damaged endothelial layer under the influence of HSV-1 in the 3rd (HSV / HFD) group. The lipid inclusions and necrotic cell debris were phagocyted by macrophages in subendothelial space of the 2nd (HFD) and 3rd (HSV / HFD) groups. However, these processes were more intense in the 3rd (HSV / HFD) group, the density of the detected phagocytes, the number and diameter of lysosomal inclusions were also greater in this group. In the 1st (HSV) group, macrophages were also detected, but their migration, in our opinion, can be associated with the elimination of cellular detritus. The latter is due to lytic changes in the cells that are induced by HSV-1, and the appearance of individual lymphocytes within the aortic wall in response to the infection.

In the third group (HSV / HFD), damage to the subintimal smooth muscle cells (edema and destruction of mitochondria, decrease in electron density and fibrous structures) was detected. We believe that in the 3rd (HSV / HFD) group ultrastructural changes progress from the aortic intima and subintimal matrix to the muscular layer. The prominent signs of necrosis of smooth muscle cells in mice after a 12-week high-fat diet were not observed (the damage was manifested only by the destruction of mitochondria).

SEM, used to evaluate the surface of the aorta, also yielded some interesting results. Prior to the experiment, we made an assumption about structural abnormalities in the aortic wall in the 2nd (HFD) group that should have been in the form of changes on inner surface of aorta, but after 12 weeks of a high-fat diet those alterations did not occur. The aortic intima had a longitudinal physiological waviness similar to that of the intact aorta of mice, as described previously [14]. But other important peculiarities were observed in infected animals. The 1st (HSV) and 3rd (HSV / HFD) groups demonstrated «abundant» adhesion of leukocytes to the aortic intima, whereas the 2nd (HFD) group showed more intense erythrocyte adhesion and swelling of the endothelial cells. Between those cells the structural defects were seen (gaps, lysis of cells with remnants of the nuclear zone of endotheliocytes). It was found that the contours of the neighboring cells did not have complete contact. The findings obtained by means of SEM correlate with TEM results and complement them. Similar gaps are described by other authors and are apparently a nonspecific manifestation of the aortic intima damage [15]. The results obtained in our study correlate with the findings that were published earlier.

CONCLUSIONS

1. The formation of atheroma in the aortic wall during a 12-week high-fat diet, as shown in the literature, was not detected.
2. Comparative analysis of the aortic changes in mice of the 1st, 2nd and 3rd groups (HSV, HFD and HSV / HFD) proves the initiation of structural changes in the aortic intima including phagocytosis of individual lipid inclusions in the subendothelial space in the 2nd (HFD) group and their subsequent accumulation in cells, cell damage and destruction, which leads to an increase in the density of lipid inclusions and cellular detritus in the extracellular aortic matrix in the 3rd (HSV / HFD) group.
3. HSV infection potentiates the accumulation of lipid inclusions in the aortic intima during a HFD, facilitates infection and contributes to the development of acute infection.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

THE RELATIONSHIP BETWEEN PROLACTIN LEVELS AND THE RESULTS OF HOLTER MONITORING IN PATIENTS WITH STABLE ANGINA OF TENSION AND GASTROESOPHAGEAL REFLUX DISEASE

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ABSTRACT

The aim: Determine the possible relationship between prolactin levels and the results of Holter monitoring in patients with stable angina of tension and gastroesophageal reflux disease (GERD).

Materials and methods: The study included 118 patients with stable angina of tension of I-II functional class. Of these, 88 patients with stable angina of tension with comorbid GERD (A, B, C), who were included in the second group of the study and 30 patients with isolated stable angina of tension which formed the 1st group. All patients who were included in the study prior to treatment were evaluated for serum prolactin levels and Holter monitoring.

Results: In patients with stable angina of tension and GERD, prolactin levels exceed the norm levels and are higher than the level of prolactin in the group with isolated stable angina ($p<0,05$), as well as the number of episodes of painful and painless myocardial ischemia and their duration ($p<0,05$). The presence of a comorbidity with GERD provokes an increase in the total duration of episodes of ischemia per day in patients with stable angina compared to those in patients with no GERD. A direct linear relationship was founded between prolactin serum levels and the number of episodes of pain in myocardial ischemia in patients with stable angina and GERD per day, and between the total duration of episodes of myocardial ischemia and prolactin levels.

Conclusions: In patients with stable angina and GERD, in 70.4% of cases, the level of prolactin exceeds the norm in 2,6 times, and is higher than the level of prolactin in the isolated group of stable angina. For the comorbidity of stable angina and GERD, the number of episodes of painful, painless myocardial ischemia and their duration is more than once in the group of patients with stable angina without GERD ($p<0.05$).

KEY WORDS: Stable angina of tension, gastroesophageal reflux disease, prolactin, Holter monitoring, stress

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INTRODUCTION

It is known that stress plays a role in the pathophysiology of chronic diseases of the gastrointestinal tract, the cardiovascular system and the endocrine system. Psychological stress is one of the factors of the onset and progression of myocardial lesions [1,2,3]. Stress is a critical factor that leads to the development of depressive disorders and hypersensitization. The influence on the development of pathological processes in the human body of chronic stress is also established. According to the conducted meta-analysis, chronic stress was found not to be a risk factor for chronic myocardial ischemia only, but also provokes the appearance of acute cardiovascular events in patients with coronary heart disease [4], causing persistent endothelial dysfunction [4]. One of the main intermediaries and indicators of both acute and chronic stress is prolactin hormone [3,4-6,7]. It is excreted in combination with other pituitary hormones in response to the influence of stress factors and performs the tread effect by changing neuronal co-operation. However, long-term increase in the level of prolactin in the bloodstream may lead to the development of pathological changes caused by hyperprolactinemia. Thus, it is reported that there is a close relationship between elevated levels of prolactin and psychological derivations in the form of the causing or increasing anxiety and irritability [7,8]. The level of prolactin above the norm promotes the development of depressive behavior [8]. Against the background of elevated levels of prolactin, the level of the vasoingibin hormone increases in the blood, which is a proteolytically cleaved prolactin that loses the ability to interact with prolactin receptors, but inhibits angiogenesis, neovascularization, narrowing lumen and vascular permeability, contributing to the onset or progression of endothelial dysfunction [8-10] Under conditions of chronic stress, elevated levels of prolactin stimulate the functional activity of fibroblasts in the myocardium, on the surface of which the receptor is expressed in the prolactin [6], which leads to the occurrence of cardiosclerosis and arrhythmias [10]. At the same time carrying out of the medical correction of hyperprolactinemia correlates with decrease in

proliferation of fibroblasts [6]. An elevated prolactin level inhibits NO-mediated vasodilation of coronary arteries due to the activation of β2-adrenergic receptors in them [11,12-14]. The inhibitory effect of hyperprolactinemia on proliferation and regenerative properties of the epithelium of the gastrointestinal tract has been established [15]. In addition, chronic stress provokes the persistence of hyperalgesia in inflamed areas of the gastrointestinal tract [4,15], which can exacerbate the manifestations of somatic pain as it stimulates perception and a sense of physical pain and discomfort behind the sternum. According to the literature analysis, the growth of prolactin levels in the range of 25-80 ng/ml is a consequence of stress induction. The level of prolactin increases in the human body under the influence of certain stress factors, so it can be considered an indicator of "chronic stress" [10,16].

THE AIM

To determine the possible relationship between prolactin levels and the results of Holter ECG monitoring in patients with stable angina and GERD.

MATERIALS AND METHODS

The study included 118 patients with stable angina pectoris of I-II functional class aged from 48 to 79 years. Of these, 88 patients with stable angina with comorbid GERD (A, B, C by the Los Angeles classification), who were included in the second group of the study and 30 patients with isolated stable angina which formed the 1st group. Diagnosis of stable angina was established on the basis of the Order of the Ministry of Health of Ukraine No. 152 of March 2, 2016 "Stable Ischemic Heart Disease". The diagnosis of GERD was established on the Lyon Consensus [17]. All patients who were included in the study prior to treatment had a determination of serum level of prolactin by immunoassay. Blood collection for determining the level of prolactin was performed in the morning, immediately after waking up from the elbow, in the minimum stressful conditions. The upper limit of the norm of prolactin level was the average value between the upper limit of the norm for men and women, which is 22.5 ng/ml. All women who participated in the study were in the post-menopausal period. With the help of daily ECG Holter monitoring (HM) results were obtained and we analyzed the total duration of myocardial ischemia (min/day), the number of episodes of painful and painless myocardial ischemia per day (n), the mean duration of episodes of painful myocardial ischemia and painless myocardial ischemia (min/day) To determine the episodes of myocardial ischemia using the Kodama criteria [2]. Other indicators that have been set up using HM ECG in this article will not be described.

Statistical processing of the obtained results was carried out using the software Microsoft Excel 2013. The distribution of samples was checked for normality using the criterion Shapiro-Wilka. The samples that had a normal distribution were checked using the parametric method, the Student's t-criterion for independent samples.

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Opinion of the Bioethical Commission on Higher State Educational Institution of Ukraine "Bukovinian State Medical University" of 3 October 2020.

RESULTS

According to the results of the study, the incidence of hyperprolactinemia in patients included in the 2nd study group was 70.4%, that is in 62 of the 88 patients with stable angina with comorbid gastrointestinal reflux disease, serum prolactin levels were found to be higher than normal. Concerning the frequency of hyperprolactinemia in patients with isolated stable angina, in 17 of 30 patients, an elevated prolactin serum level was detected at 56%. By analyzing the results of the prolactin serum levels in both groups of the study, it was found that in the case of an isolated course of stable angina, and with the comorbid flow of stable angina with GERD, the mean value of prolactin level in each group exceeded the norm. However, with the combined course of stable angina and GERD, a significantly higher prolactin level was observed than in the first group, which exceeded the norm by 2.6 times ($p<0,05$) (Tab I). In patients of the 1st group, the average prolactin level exceeded the norms in 1.6 times ($p<0.05$) (Tab I). Comparing the mean value of the prolactin level of both groups, it was found that in the conditions of the combined course of stable angina and GERD is 1.7 times higher than in the case of an isolated course of stable angina, which is a significant statistical difference ($p<0.05$) (Tab I).

The transient increase in prolactin levels is a physiological response to a stress factor, but if the factor is constant or repetitive, the level of prolactin may remain for a long time at a high level, because of the depletion of the inhibitory mechanisms that is controlling the levels of prolactin [10,16]. In our opinion, an increase in its level in the blood of patients with stable angina is due to the constant presence of patients in conditions such as psychological (awareness of the presence of the disease, the need for constant intake of medication, regular visits to the district therapist and / or cardiologist, the need for constant control of the disease and the fear of the next chest pain and the fear of death), genetic stress, and physical stress (pain behind the sternum). Under the conditions of the comorbidity of stable angina with GERD, the number of both psychological (awareness of the presence of 2 pathologies, disturbing and obsessive thoughts about the possible iatrogenic effect of drugs, the reception of a large number of medications) and physiological stressors increases due to clinical manifestations of the disease (heartburn, acid regurgitation, dysphagia, odynophagia, pain behind the sternum, which can mimic an attack of angina etc.). Actually, all of the above and leads to chronic stress and hyperprolactinemia. According to Holter's ECG monitoring in the 2nd study group, the mean value of the number of episodes of pain in myocardial ischemia exceeded the similar indicator of

Table I. Levels of prolactin and ECG Holter monitoring in patients of both groups of study (M ± m)

Indicator	Unit	1st Group stable angina only (n=30)	2nd Group stable angina + GERD (n=88)
Prolactin	ng/ml	35,63±2,92	58,3±2,42*
Total duration of MI	min/day	25,76±1,93	34,96±1,36*
Number of painful MI episodes	n	2,96±0,33	4,59±0,19*
Number of painless MI episodes		5,61±0,3	7,75±0,36*
Average duration of painful MI	min/day	5,25±0,48	7,03±0,44*
Average duration of painless MI		4,47±0,85	6,23±0,47*

* - the difference is reliable, compared with the results of patients in the first group.

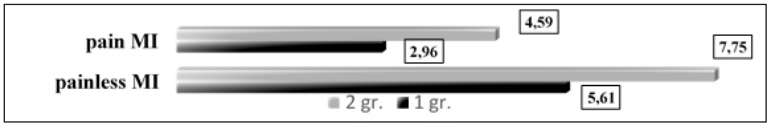

Fig 1.
HM ECG - Holter monitoring of electrocardiography;
GERD - gastroesophageal reflux disease

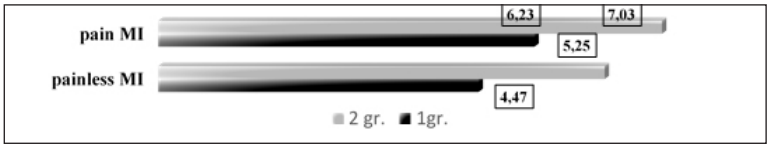

Fig 2.
HM ECG - Holter monitoring of electrocardiography;
GERD - gastroesophageal reflux disease

patients with isolated stable angina by 1.5 times (p<0.05) (Tab I, Fig 1), which can testify to the provoking effect of the inflammatory process of the lower esophagus on the myocardium, under the conditions of the comorbidity of stable angina with GERD. At the same time, the average duration of myocardial ischemia was 1.4 times higher compared with patients in the 1st group (Tab I), (Fig. 2). A similar trend was also observed in the analysis of the average number of episodes of painless myocardial ischemia (Table I, Fig. 1). So the number of episodes in the group of patients with stable angina and GERD exceeded that in the group of patients with isolated stable angina in 1,3 times (p<0,05) (Fig 1), as well as the mean duration of painless myocardial ischemia in 1,2 times (p <0,05) (Tab I), (Fig 2).

By conducting an in-depth analysis of the results of the second group patient study, our attention was also drawn to the fact that serum prolactin levels and the number of episodes of pain in myocardial ischemia are depended. It was found that the value of the Pearson correlation coefficient (r) for these indices is 0.34 (p<0,05), which indicates direct linear relationship between the level of serum prolactin and the number of episodes of pain in myocardial ischemia. The mean interaction correlation (r=0.25) was determined by an analysis between the level of prolactin and the number of episodes of painless myocardial ischemia. Correlation between the duration of pain or painless myocardial ischemia and the prolactin level in patients in group 1 was within the range of weak (r=0.15 and r=0.21) (p<0.05), in contrast to the above described correlation in 2nd study group. Thus, in patients with ischemic heart disease and GERD, the highest level of prolactin serum was detected suggested the greater number of episodes of pain in myocardial ischemia was recorded in patients with HM.

DISCUSSION

In our opinion, the proportional increase in the prolactin level and the number of episodes of pain in the course of the day is due to the presence of patients in conditions of constant stress, which is caused by frequent attacks of remitting pain, which occurs as a result of episodes of myocardial ischemia, and as a result of GERD symptoms. After all, the pain behind the sternum due to gastroesophageal reflux can simulate the clinic of coronary abdominal pain, which leads to a constant fear of a possible occurrence of angina, anxiety and "worst" expectations. Concerning the total duration of episodes of ischemia per day, it was found that this rate of patients in group 2 was 1.4 times that of a group of patients with isolated stable angina (Tab I). This may indicate that the presence of comorbid GERD not only can provoke angina attacks [18], but also affect their duration. We found a correlation relationship (r=0.37, p<0.05)) between the total duration of episodes of MI per day and the level of serum prolactin. Consequently, the presence of chronic chest pain due to GERD in patients with stable angina leads to an increase in the level of total MI per day, which is in close correlation with the level of serum prolactin.

CONCLUSIONS

In patients with stable angina and GERD, in 70.4% of cases, the level of prolactin exceeds the norm in 2,6 times, and is higher than the level of prolactin in the isolated group of stable angina. For the comorbidity of stable angina and GERD, the number of episodes of painful, painless myocardial ischemia and their duration is more than once in the group of patients with stable angina without GERD (p<0.05). There is a direct linear relationship between

serum prolactin levels and the number of episodes of painful myocardial ischemia per day in patients with stable angina and GERD. The presence of a comorbid GERD in patients with stable angina leads to an increase in the total duration of episodes of ischemia per day in patients with stable angina compared to patients who have no GERD. We have established correlation between the total duration of episodes of myocardial ischemia and the level of serum prolactin in the blood in patients with stable angina of tension under conditions of comorbid GERD.

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ORIGINAL ARTICLE
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CHANGES IN THE LEVEL OF α1-ANTITRYPSIN IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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ABSTRACT

The aim: To investigate changes in the level of α1-antitrypsin (A1AT) in blood and in stool and their diagnostic value in patients with NAFLD and with impaired carbohydrate metabolism at different stages of liver damage.

Materials and methods: 34 patients with non-alcoholic fatty hepatosis (NAFH) and 40 patients with non-alcoholic steatohepatitis (NASH) were examined. Enzyme-linked immunosorbent assay for all patients with serum and coli was performed by determining the level of A1AT.

Results: In patients with NAFLD, in combination with IR, the level of A1AT in the blood plasma is only 1.6 times higher than that of the control group ($p < 0.05$), and in the case of combination of NAFH and type 2 diabetes mellitus (T2DM) – it is 2 times higher ($p < 0.05$). In patients with NASH in combination with insulin resistance (IR), the level of A1AT in the serum was 4.1 times higher than in the control group, and for NASH and type 2 diabetes – 4.2 times.

Conclusions: The level of A1AT in the blood plasma increases proportionally to the degree of progression of liver damage (from the minimum values at NAFH to the maximum at NASH), regardless of the type of carbohydrate metabolism disturbance. The combination of NAFLD and T2DM is accompanied by a more pronounced increase in A1AT in stool and α1-antitrypsin clearance than the combination of NAFLD and IR.

KEY WORDS: non-alcoholic fatty liver disease type, 2 diabetes mellitus, insulin resistance, α1-antitrypsin

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is prevalent worldwide and is the most common chronic liver disease in Western countries. Its increasing prevalence is associated with major risk factors such as obesity, dyslipidemia, type 2 diabetes mellitus (T2DM), and the metabolic syndrome [1]. NAFLD is a systemic disorder of energy, glucose, and lipid homeostasis with hepatic manifestations [2]. Metabolic disorders, such as lipid accumulation, insulin resistance (IR), and inflammation, have been implicated in the pathogenesis of NAFLD, but the underlying mechanisms, including those that drive disease progression, are not fully understood [3].

Alpha-1 Antitrypsin (A1AT) is a highly polymorphic serum protein. Several genetic variants are associated with varying degrees of decreased serum levels; however, these levels can rise in response to infection, inflammation, injury and estrogen levels. Although the effect of inflammation is well established, it has never been studied quantitatively with respect to specific genotypes in a large representative sample [4]. A1AT is a circulating liver derived protease inhibitor [5]. Despite A1AT well-known involvement in hepatic fibrosis, its role in NAFLD pathogenesis is not well characterized.

THE AIM

To investigate changes in the level of α1-antitrypsin in blood and in stool and their diagnostic value in patients with NAFLD and with impaired carbohydrate metabolism at different stages of liver damage.

MATERIALS AND METHODS

At the clinical site of the department of propaedeutic of internal diseases of the medical faculty of SHEI "UzhNU" (gastroenterology and endocrinology department of the TRCH n. a. A. Novak) during 2016-2019, 74 patients with NAFLD were examined. The average age was 47.8 ± 7.9 years. Patients were divided into Groups, namely:

- Group I included 34 patients with non-alcoholic fatty hepatosis (NAFH) (among them there were 20 males (58.8%) and 14 females (41.2%), the average age was 47.7 ± 6.8 years)
- Group II included 40 patients with non-alcoholic steatohepatitis (NASH) (among them there were 24 males (60.0%) and 16 females (40.0%), the average age was 48.9 ± 6.8 years)

In order to achieve the aim of the research, the patients were further divided into subgroups depending on the presence of type 2 diabetes (moderately severe) or insulin resistance (IR).

The NAFH group (n = 34) was divided as follows: 1.1 had 16 patients with insulin resistance (IR) and 1.2 had 18 patients with type 2 diabetes (T2DM).

Group II (n = 40) with NASH patients was divided as follows: 2.1 included 20 patients with insulin resistance (IR) and 2.2 had 20 patients with type 2 diabetes (T2DM).

The control group included 20 practically healthy persons (12 males (60.0%) and 8 females (40.0%)), the average age was 47.6 ± 5.8 years.

All studies were performed with patients consent (written consent for performing appropriate diagnostic and therapeutic measures was received from all of the patients), and the methodology of their implementation were in line with the Helsinki Declaration of Human Rights of 1975 and its 1983 revised version, and in line with Europe Convention on Human Rights, as well as biomedicine and legislation of Ukraine.

All the examined patients were subject to general clinical, anthropometric, instrumental, and laboratory tests. To verify the diagnosis, attention was paid to the nature of complaints, as well as the history of the disease. In anthropometric study, height, weight, waist circumference were determined, and body mass index (BMI) was calculated.

Ultrasound examination of the abdominal cavity was performed on all patients according to generally accepted method. Standard and biochemical blood serum tests have been performed to determine the functional state of the liver, lipid metabolism indexes and carbohydrate metabolism (glucose, insulin, glycosylated hemoglobin (HbA1c, %) indexes.

NAFLD diagnosis was established in accordance with the Unified Clinical Protocol criteria (Order of the Ministry of Health of Ukraine dated November 6, 2014, No. 826) and EASL-EASD-EASO Clinical Recommendations for Diagnosis and Treatment of NAFLD [6].

The degree of liver damage has been calculated using surrogate markers of fibrosis with the help of online calculators: NAFLD fibrosis score (NFS), Fibrosis 4 calculator (FIB-4), Fibrotest.

Insulin resistance was determined using the HOMA-IR (homeostasis model assessment method for insulin resistance), which was calculated according to this formula (normally HOMA-IR – < 2.5):

HOMA = blood insulin serum on an empty stomach (μU/mL) × blood plasma glucose on an empty stomach (mmol/L) /22.5

Diagnosis of type 2 diabetes mellitus has been established according to IDF recommendations (2005), as well as taking into account the criteria of unified clinical protocol (Order of Ministry of Health of Ukraine dated December 21, 2012, No. 1118) [7]. Level of severity of T2DM was evaluated according to the HbA1c (norm being up to 6.0%).

Enzyme-linked immunosorbent assay for all patients with serum and coli was performed by determining the level of α1-antitrypsin, using the test system of Immundiagnostic AG (Germany). Clearance of α1-antitrypsin was calculated based on the results of the assay.

The criteria for exclusion of patients from the study were also: type 1 diabetes, type 2 diabetes (moderate to severe degrees, with severe manifestations of diabetic angioneuropathy), chronic hepatitis, alcoholic, viral (hepatitis B, C, D) etiologies, autoimmune hepatitis.

The analysis and processing of the patient examination results were carried out using the computer program "Statistics" for Windows v.10.0 (StatSoft Inc., USA) using parametric and nonparametric methods of evaluating the results obtained.

RESULTS

It should be noted severe disruptions of carbohydrate metabolism when combined with type 2 diabetes or IR with NAFLD. In this case, the maximum deviations from the parameters of the control group is established in patients Subgroups 1.1 (NAFH in combination with IR) – tables I.

Changes of α1-antitrypsin in patients with NAFLD by subgroups depending on the type of carbohydrate metabolism disorders are presented in table II.

The results of our study showed that in patients with NAFLD, the index of α1-antitrypsin in blood plasma is significantly increased, compared with its level in healthy persons. It should be noted that in patients with NAFLD, in combination with IR, the level of A1AT in the blood plasma is only 1.6 times higher than that of the control group ($p < 0.05$), and in the case of combination of NAFH and type 2 diabetes – it is 2 times higher ($p < 0.05$). In patients with NASH in combination with IR, the level of A1AT in the serum was 4.1 times higher than in the control group ($p < 0.01$), and for NASH and type 2 diabetes – 4.2 times ($p < 0.01$).

When characterizing changes in A1AT levels in stool, in the patients we examined, we found more pronounced deviations from the norm when combining NAFLD and type 2 diabetes. Specifically, in patients in the 1.2 subgroup this indicator was increased to 30.90±0.76 mg / dL ($p < 0.05$), and in patients in the 2.2 subgroups – to 45.7 ± 1.11 mg / dL ($p < 0.05$).

Note the marked increase in A1AT clearance in patients with NASH and type 2 diabetes (up to 122.14 ± 8.23 ml / day – $p < 0.001$), as well as with NAFLD and type 2 diabetes (up to 88.16 ± 0.45 ml / day – $p < 0.01$). In patients with IR and liver damage, the clearance of A1AT was lower in both subgroups.

DISCUSSION

Therefore, in patients with NAFLD, both at the stage of NAFH and at the stage of NASH, an increase in the level of A1AT in the serum was established. It is known that A1AT is an inhibitor of proteolytic enzymes (trypsin, chymotrypsin, elastase, kallikrein, catapsin), and also indicates an inflammatory process in the body, including the liver. This is evidenced by a more pronounced increase in its level in patients examined at the stage of NASH.

Increased intestinal A1AT loss when combined with NAFLD with type 2 diabetes may indicate impaired intestinal mucosal permeability in these patients and indicate a systemic nature of the disease. Our results require further studies to understand the processes that occur in patients with combination of NAFLD and impaired carbohydrate metabolism. In the professional literature, the issue of the influence of disorders in the gut microbiocenosis on the formation of metabolic

Table I. Changes of carbohydrate metabolism parameters in patients with NAFLD depending on presence of T2DM or IR

Indicator	Control group	Participants in main with NAFLD			
		Group I (n=34)		Group II (n=40)	
		Subgroup 1.1 (n=16)	Subgroup 1.2 (n=18)	Subgroup 2.1 (n=20)	Subgroup 2.2 (n=20)
Glucose (mmol/L)	4.51 ± 0.41	6.95±0,27*	7.95±0.24*	6.75±0.31*	7.87±0.19*
HbA1c, %	4.38 ± 0.26	6.88±0,36*	7.98±0.36*	6.80±0.36*	7.89±0.23*
Insulin (U/L)	9.20 ± 1.14	44.7±6,5**,+	29.7±3.3**	38.4±4.1**,+	21.3±1.7*
C-peptide (ng/mL)	4.54 ± 0.97	21.77±2,31**	14.56±1.45**,+	17.41±1.67**	12.33±1.22**,+
HOMA-IR	1.71 ± 0.32	12.81±3,26**	10.41±2.9**	11.32±2.78**,+	7.44±0.23**

Note: the difference between the indicators in patients of Group 1 and control group is significant - * - p <0.05; ** - p <0.01; the difference between the indices in patients by subgroups is significant - + - p <0.05; ++ - p <0.01.

Table II. The content of α1-antitrypsin in blood plasma and in stool samples and its clearance in patients with NAFLD

Patient groups	Рівень α1-antitrypsin (mg/dl)		α1-antitrypsin clearance (mL/24hrs)
	in blood plasma	in stool	
Control group	128.12±1.23	15.83±0.19	18.77±0.65
I group (n=34) subgroups:			
Subgroup 1.1 (n=16)	203.05±4.49*	18.26±1.02	21.15±0.88
Subgroup 1.2 (n=18)	256.17±4.45*	30.90±0.76*	88.16±0.45**
II group (n=40) subgroups:			
Subgroup 2.1 (n=20)	527.16±6.16**	21.83±0.71	44.21±2.18*
Subgroup 2.2 (n=20)	544.08±9.15**	45.7±1.11*	122.14±8.23***

Note: statistically significant difference was found between control group and patient groups: * - p <0.05; ** - p <0.01; *** - p <0.001

diseases, including type 2 diabetes, IR, and NAFLD, is actively discussed. In our opinion, assessment of the barrier function of the intestine for its adequate correction, as well as therapy aimed at reducing inflammation in the liver and apparently in the intestine, should be a major element in the prevention of the progression of liver damage in its fatty disease and its combination with impaired carbohydrate metabolism .

CONCLUSIONS

1. In patients with NAFLD, increased levels of α1-antitrypsin in blood plasma and in stool have been established
2. The level of A1AT in the blood plasma increases proportionally to the degree of progression of liver damage (from the minimum values at NAFH to the maximum at NASH), regardless of the type of carbohydrate metabolism disturbance (IR, T2DM).
3. The combination of NAFLD (NAFH, NASH) and type 2 diabetes is accompanied by a more pronounced increase in A1AT in stool and α1-antitrypsin clearance than the combination of NAFLD and IR.

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D – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE
PRACA ORYGINALNA

SUBSTANTIATION OF DIAGNOSIS AND TREATMENT OF CHRONIC RECURRENT APHTHOUS STOMATITIS IN CROHN'S DISEASE

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ABSTRACT

The aim: To substantiate the diagnosis and treatment of chronic recurrent aphthous stomatitis in Crohn's disease.

Materials and methods: The analysis of diagnostic and treatment of 52 patients with chronic recurrent aphthous stomatitis in Crohn's disease (main group), mean age 31.8 + 2.3 was performed. The comparison group consisted of 50 patients with chronic recurrent aphthous stomatitis not associated with Crohn's disease (mean age 34.7 + 1.8). Patients in both groups were studied for clinical manifestations, morphological and immunohistochemical studies the aphthae on the oral mucosa were performed.

Results: An objective evaluation of the oral mucosa showed that the aphthae on the oral mucosa in patients of both groups did not differ visually. In the morphological study, the patients in the main group had granulomatous inflammation of the oral mucosa, characteristic of Crohn's disease, while the patients in the comparison group had fibrinous inflammation. Immunohistochemical study of the cell infiltrate phenotype in the area of the lesion revealed that the patients in the main group there prevailed CD68+macrophages, the appearance of both intraepithelial and cell infiltrates of T-lymphocyte suppressors, which is characteristic of Crohn's disease.

Conclusions: Diagnosis of chronic recurrent aphthous stomatitis in Crohn's disease is based on biopsy of the aphthae on the oral mucosa and their morphological examination, the results of which confirm the presence of granulomatous inflammation, with a large number of macrophages, the presence of T-lymphocytes, characteristic of Crohn's disease, while recurrent aphthous stomatitis of another genesis morphologically detect fibrinous inflammation.

The choice of therapeutic tactics for chronic recurrent aphthous stomatitis depends on the results of the morphological study. When granulomatous inflammation is detected in patients with chronic recurrent aphthous stomatitis, which is characteristic of Crohn's disease, in addition to topical treatment of the oral mucosa, specific therapy with mesalazine drugs is prescribed.

KEY WORDS: aphthous stomatitis, Crohn's disease, granulomatous inflammation

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INTRODUCTION

Chronic recurrent aphthous stomatitis is an inflammation of the oral mucosa, manifested by the presence of aphthae, which is characterized by a prolonged course, frequent relapses without a certain pattern and often is accompanied by the gastrointestinal tract diseases [1,2]. Chronic recurrent aphthous stomatitis is observed in 11.5% of patients with Crohn's disease [3]. Diagnosis of chronic recurrent aphthous stomatitis in Crohn's disease in the absence of intestinal manifestations is often limited only by objective changes on the part of the oral mucosa [4].

Biopsy of the aphthae of the oral mucosa with morphological examination for the purpose of establishing a precise diagnosis is usually not performed [5]. The precise diagnosis of chronic recurrent aphthous stomatitis in Crohn's disease is established rather late, respectively, patients receive only local treatment, and specific treatment is not prescribed. The results of the treatment of chronic recurrent aphthous stomatitis in Crohn's disease should also be considered unsatisfactory, as the frequency of remissions and recurrences is quite high [6,7]. In our opinion, biopsy of the oral mucosa with morphological examination in chronic recurrent aphthous stomatitis will improve the diagnostic results and substantiate therapeutic tactics.

THE AIM

To substantiate the diagnosis and treatment of chronic recurrent aphthous stomatitis in Crohn's disease.

MATERIALS AND METHODS

The analysis of diagnostic and treatment of 52 patients with chronic recurrent aphthous stomatitis in Crohn's disease (main group) was performed. The age of patients was 18 to 65 (mean age 31.8 $\pm$ 2.3), among them the number of women was 29 (55.8%) and men – 23 (44.2%). The comparison group consisted of 50 patients with chronic recurrent aphthous stomatitis which was not associated with Crohn's disease. Age of patients from 18 to 65 years (mean age 34.7 $\pm$ 1.8), among them the number of women was 31 (62%) and men – 19 (38%).

Diagnosis of chronic recurrent aphthous stomatitis in patients in both groups included examination of complaints, history, objective examination of the oral mucosa and biopsy with morphological examination of the aphthae. The biopsy was performed on patients of both groups with their consent and according to the positive decision of the Bioethics Committee of Shupyk National Medical Academy of Postgraduate Education (Protocol No. 1 dated 03.01.2017). Under infiltration anesthesia, the aphthae on

the oral mucosa with a conchotome were taken with tissue pieces 4 – 5 mm in size. The obtained biopsies were fixed in 10% solution of neutral buffered formalin (pH 7.4) for 24-36 hours, the material was prepared out according to the conventional method, it was poured into paraffin. From paraffin blocks on a rotary microtome NM 325 (Thermo Shandon, England) serial histological sections with a thickness of 4-5 μm were cut, which then were stained with H&E, the PAS reaction [8,9].

The immunohistochemical study was conducted to determine the phenotype of cellular infiltrate [10,11]. For immunohistochemical study, sections were placed on Super Frost Plus adhesive glasses (Menzel, Germany). Citrate buffer (pH6), EDTA buffer, pH8 were used for high-temperature treatment of antigen epitopes. We used antibodies and an UltraVision Quanto HRP detection system, a Quanto DAB chromogen, manufactured by Thermo Fisher Scientific (the USA). Monoclonal murine antibodies to CD68/macrophage marker (KP1 clone), monoclonal rabbit antibodies to CD8+/ marker T-lymphocyte suppressors (3B5). Pathomorphological examination and photoarchiving were performed using ZEISS (Germany) optical microscopes with «Axio Imager. A2» data processing system with 5x, 10x, 20x, 40x, 1.5 binoculars and 10 eyeglasses with ERc 5s camera, «Carl Zeiss» Primo Star with Axiocam105 color camera.

All patients underwent topical treatment using anti-inflammatory, antiseptic, analgesic, keratoplastic drugs. After obtaining the results of morphological examination the aphthae on the oral mucosa, the treatment was reviewed and supplemented as necessary.

RESULTS

Patients in the main group and the comparison group had such major complaints as one or more aphthae of the oral mucosa, pain during meals and conversation. In 7 (13.5%) patients of the main group, besides manifestations from the oral cavity, there were complaints from the intestine, namely abdominal pain and recurrent diarrhea.

The results of an objective study in patients in both groups were comparable. The aphthae had an oval or circular shape, with a diameter of 6 ± 3.2 mm, surrounded by an inflammatory rim of red color, covered with fibrinous plaque, around the aphtha – infiltration of the mucous membrane. Palpation is very painful, mild.

The results of morphological study of biopsies in patients of the main group showed the presence of expressed degenerative changes in mucous membranes in the areas of the lesions, mainly in the form of vacuolar and/or balloon dystrophy in the epithelium, areas of parakeratosis, focal manifestations of inflammatory reaction in the form of intraepithelial leukocytes, cells with signs of apoptosis, focal acanthosis. In the submucosal layer, there was a pronounced, mainly limited by the type of granulomatous inflammation, lymphohistiocytic cell infiltrate, which spread on separate areas to the epithelial layer. Signs of dysmucoidosis were observed in the stroma of the site of focal sclerosis in the vessels of the microcirculatory bloodstream, in some part – sclerosis (Figure 1).

To determine the phenotype of cellular infiltrate in the lesion sites the immunohistochemical study was conducted. In areas of cellular infiltration in the form of granulomatous inflammation, a marked positive expression of a large number of CD68+macrophages was observed (Figure 2).

In addition, we found that among the cells of inflammation there were present T-lymphocyte suppressors, which were localized, both intraepithelially and subepithelially in the area of cellular infiltrate in the stroma (Figure 3). Such a morphological pattern corresponds to Crohn's disease. The diagnosis of Crohn's disease in the patients of the main group was also confirmed by a gastroenterologist and the results of ileocolonoscopy, biopsy of the mucous membrane of the distal ileum with morphological examination.

In the pathomorphological study of biopsies in patients of the comparison group we found mainly fibrinous and fibrinous-necrotic type of inflammation of the mucous membrane – in the form of ulcers and films of fibrin on the surface, which was accompanied by a sharp decrease in mucus secretion in these areas. Cell inflammatory infiltrate at the bottom and at the edges of the ulcers is mainly represented by neutrophilic leukocytes, monocytes, focal lymphocytes. In the epithelium, along with the alterative-exudative changes there is observed edematous layer of the epithelium, focal spongiosis, which can lead to the formation of microvavities. Areas of ulcer – the defect of the epithelium is gradually filled by fibrinous exudate, which is strongly bound to the adjacent soft tissues (Figure 4).

In the areas near the defects there is preserved multilayered flat epithelium with degenerative changes, subepithelially irregular mostly moderately expressed lympho-histio-plasmacytic cell infiltration, including around the vessels of the microcirculatory bloodstream (Figure 5).

During immunohistochemical study in the ulceration area macrophage expression was not observed, outside the sites of alterative-exudative changes macrophages were found subepithelially in the stroma in the cell infiltrate with less pronounced positive expression and in a smaller number than in the main group (Figure 6).

T-lymphocyte suppressors were found outside the areas of ulceration, both intraepithelially and in areas of cellular infiltration, but their number and expression level were smaller than in the main group (Figure 7).

The results of the pathomorphological study served as the basis for the differential approach in the choice of therapeutic tactics in patients of the two groups. Patients in the comparison group who were found to have morphologically fibrinous inflammation, with areas of ulcer characteristic of chronic recurrent aphthous stomatitis, were prescribed topical treatment, which included irrigation of the oral mucosa with anti-inflammatory, antiseptic, analgesic drugs, anti-inflammatory and keratin applications. Patients in the main group who had granulomatous inflammation, characteristic of chronic recurrent aphthous stomatitis in Crohn's disease, were prescribed specific therapy with mesalazine for 1 month, in addition to topical treatment of aphthae on the oral mucosa.

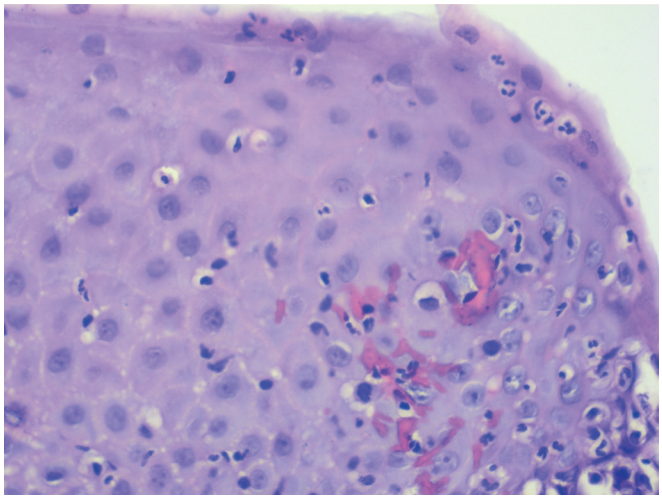

Figure 1. Expressed degenerative changes in the mucous membrane, areas of parakeratosis, accumulation of intraepithelial leukocytes, cells with signs of apoptosis. Hematoxylin and eosin staining, x400.

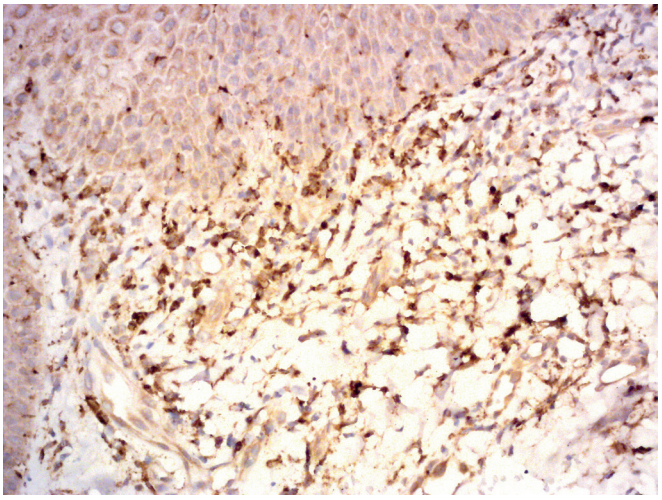

Figure 2. Area of cell infiltrate by granulomatous inflammation type, large number of macrophages with pronounced expression. Immunohistochemical study with monoclonal antibodies to CD68, x200.

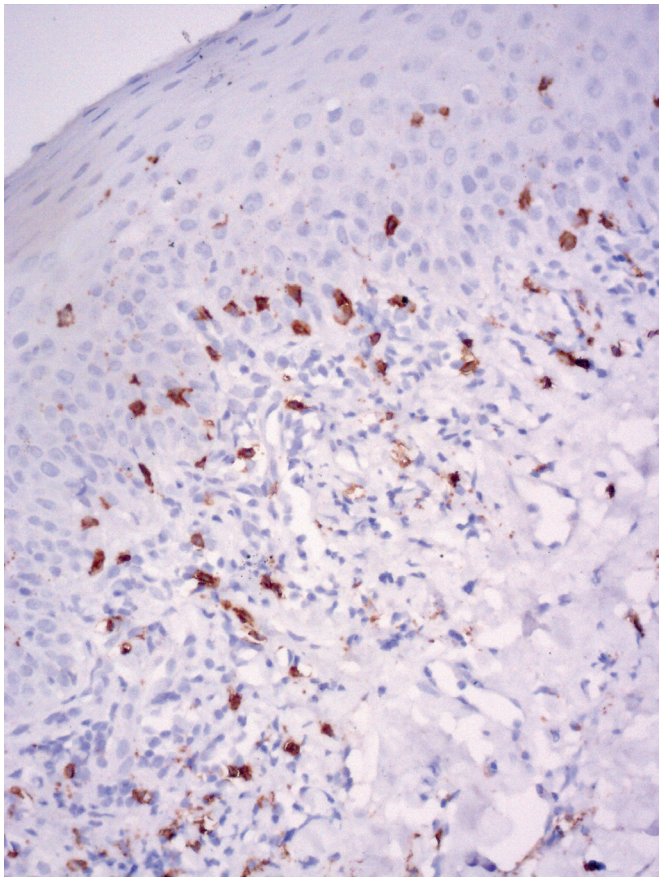

Figure 3. Pronounced positive expression of T-lymphocyte suppressors intraperitoneally, in the area of cellular infiltrate. Immunohistochemical study with monoclonal antibodies to CD8+, x200.

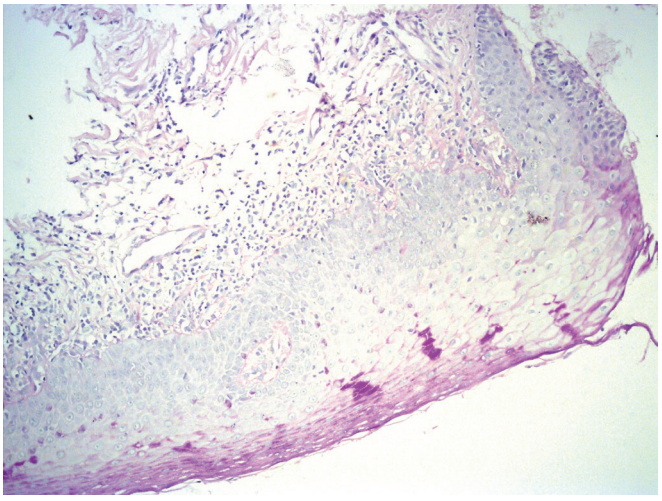

Figure 4. Ulcers, fibrin on the surface, a sharp decrease in mucus production, focal spongiosis, leukocyte cell infiltrate. PAS, x100.

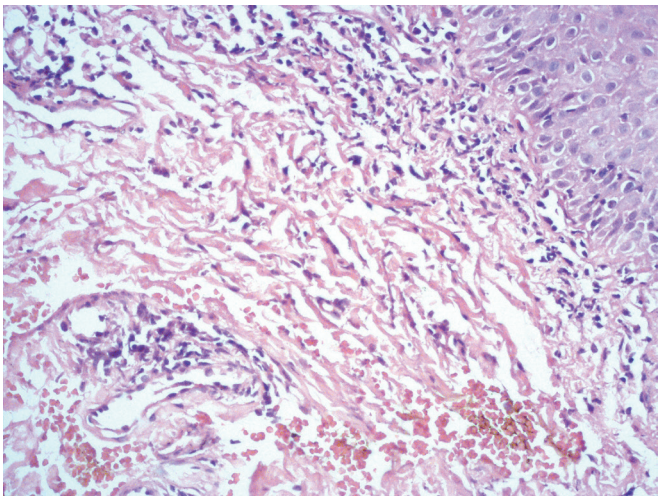

Figure 5. Lympho-histio-plasmacytic, perivascular cell infiltration is moderately expressed in the stroma. Hematoxylin and eosin staining, x200.

DISCUSSION

The results of a study of clinical manifestations of chronic recurrent aphthous stomatitis in the main and comparison groups of patients indicate that they are similar and differ only in 13.5% of patients in the main group, who

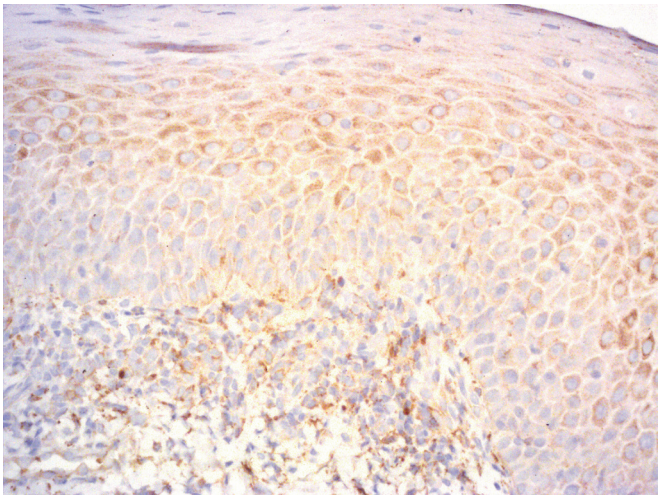

Figure 6. Poorly pronounced expression of macrophages, mainly subepithelially in the stroma. Immunohistochemical study with monoclonal antibodies to CD68+, x200.

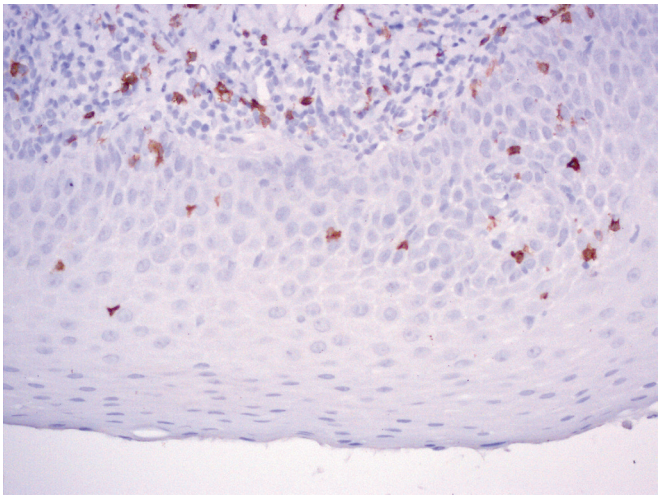

Figure 7. Moderately pronounced positive expression of T-lymphocyte suppressors intraepithelially, in the stroma subepithelially in the cell infiltrate. Immunohistochemical study with monoclonal antibodies to CD8 +, x200.

had a combination of clinical symptoms of oral cavity with intestinal disorders. Objective evaluation of the oral mucosa showed that aphthae on the oral mucosa in patients in the main group and comparison group were not visually different. Such a clinical picture does not allow us to determine the nature of inflammation and accordingly to appoint adequate treatment. The results of morphological examination of the aphthae showed the difference in inflammation of the oral mucosa in the patients of the main group and the comparison group. Granulomatous inflammation of the oral mucosa characteristic of Crohn's disease was found in patients in the main group, whereas fibrinous inflammation was found in the comparison group. Immunohistochemical study of the cell infiltrate phenotype in the area of the lesion revealed that in the patients of the main group there prevailed macrophages, the appearance of both intraepithelial and cell infiltrates of T-lymphocyte suppressors, which is characteristic of Crohn's disease. This made it possible to review the treatment, namely the patients of the main group, to prescribe, in addition to topical treatment, mesalazine group drugs for 1 month, which lead to a stable remission of 12 ± 1.2 months. This was possible only due to a morphological study of the oral mucosa, which helped establish the nature of inflammation.

CONCLUSIONS

Diagnosis of chronic recurrent aphthous stomatitis in Crohn's disease is based on biopsy of the mucous membranes of the oral cavity and their morphological studies, the results of which confirm the presence of granulomatous inflammation, with a large number of macrophages, the presence of T-lymphocyte suppressors, characteristic of Crohn's disease, while fibrinous inflammation is morphologically detected in chronic recurrent aphthous stomatitis of other genesis.

The choice of therapeutic tactics in chronic recurrent aphthous stomatitis depends on the results of the morphological study. When granulomatous inflammation is detected in patients with chronic recurrent aphthous stomatitis, which is characteristic of Crohn's disease, in addition to topical treatment of the oral mucosa, specific therapy with mesalazine drugs is prescribed.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

PERIODONTAL STATUS IN PATIENTS WITH DISEASES OF HEPATOBILIARY SYSTEM, BURDENED WITH TOBACCO AND DRUG ADDICTION

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ABSTRACT

The aim: To study the effect of drug addiction and smoking on the status of periodontal tissues in patients with hepatobiliary pathology.

Materials and methods: 58 smokers, 63 drug addicts with hepatobiliary pathology were examined and 92 persons of comparison group (with inflammatory periodontal diseases affected by hepatobiliary pathology, without addiction). Examination of patients included determination of iodine number by Svrakov, S-L and Stallard hygiene indices, PMA index and index PBI.

Results: The results of examination of smokers are worse in patients with cirrhosis than in patients with chronic toxic hepatitis and non-alcoholic steatohepatitis and also the results of these two groups was significantly worse than that of the comparison group (iodine number by Svrakov – 1,4 times and 1,3 times higher, PMA index – 1,7 times and 1,4 times worse, in accordance). The results of the study showed that drug addicts patients are worse values in patients with cirrhosis than in patients with chronic toxic hepatitis and non-alcoholic steatohepatitis and also the results of these two groups was significantly worse than that of the comparison group (iodine number by Svrakov – 1,5 times and 1,4 times higher, PMA index – 1,7 times and 1,5 times worse, in accordance).

Conclusions: Presence of hepatobiliary pathology in smokers and drug addicts increases the risk of periodontal diseases.

KEY WORDS: inflammatory periodontal diseases, hepatobiliary pathology, tobacco smoking, drug addiction

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INTRODUCTION

There is a clear trend to the increase in the number of patients with chronic liver disease, where the presence of numerous harmful habits (smoking, addictive and toxic substances) is a confounding factor [1, 2, 3, 4]. Hepatobiliary system is also adversely affected by numerous pathogenic factors of endogenous origin, leading to impaired detoxification function with subsequent development of endotoxicosis [5, 6, 7, 8, 9].

The relationship between hepatobiliary system diseases and dental pathology is conditioned by impaired barrier and antimicrobial functions of the liver, resulting in translocation of opportunistic pathogenic bacteria into organs and tissues of oral cavity [10, 11]. History, aggravated with drug addiction and harmful habits, namely smoking, usually exacerbates manifestations of the underlying disease, and its clinical course becomes longer. These factors create obstacles to the quality of dental care [2, 4].

THE AIM

To study the effect of drug addiction and smoking on the status of periodontal tissues in patients with hepatobiliary pathology.

MATERIALS AND METHODS

121 persons, including 58 smokers (suffering from chronic toxic hepatitis (22 persons), steatohepatitis (22 persons), nonalcoholic steatohepatitis (20 persons), liver cirrhosis (16 persons), and 63 drug addicts (suffering from chronic toxic hepatitis (21 persons), nonalcoholic steatoSurveys have shown that drug use causes the development of diseases of GBS and impedes the development of pathology of periodontal tissues. Also, smoking worsens the course of GBD and periodontal tissue disorders.hepatitis (22 persons), liver cirrhosis (20 persons) were examined. Patients of both sexes aged 20–45 years were included into groups under study in order to investigate peculiarities of the status of periodontal tissues.

Examination of patients included history taking, objective examination of oral cavity, determination of iodine number by Svrakov, S-L and Stallard hygiene indices, PMA index and bleeding index (PBI).

The comparison group included 92 persons with inflammatory periodontal diseases affected by hepatobiliary pathology, without tobacco and drug addiction.

Patients of all groups under study were informed, and measures were taken for patient's health safety, observance of rights, human dignity and moral and ethical standards

Table I. Index evaluation of the status of periodontal tissues in tobacco-dependent patients against the background of hepatobiliary system pathology

Groups / Indices	Comparison group (patients with inflammatory diseases of periodontium) n=92	Smokers with chronic toxic hepatitis n=22	Smokers with nonalcoholic steatohepatitis n=20	Smokers with liver cirrhosis n=16
Iodine number by Svrakov, score	1,72±0,02	2,15±0,08*	2,29±0,05*	2,48±0,03*
S-L, score	1,21±0,02	2,17±0,04*	2,20±0,03*	2,32±0,02*
Stallard, score	1,25±0,01	2,15±0,04*	2,18±0,03*	2,29±0,02*
PMA, %	40,77±0,60	59,31±1,73*	61,32±0,78*	67,8±1,18*
PBI, score	1,21±0,03	1,72±0,08*	2,01±0,04*	2,23±0,03*

Note:
* – probability index (p<0,05) compared to comparison group;
# – probability index (p<0,05) compared to group of smokers with liver cirrhosis.

Table II. Index evaluation of the status of periodontal tissues in drug-addicted patients against the background of hepatobiliary system pathology

Groups / Indices	Comparison group (patients with inflammatory diseases of periodontium) n=92	Drug-addicted patients with chronic toxic hepatitis n=21	Drug-addicted patients with nonalcoholic steatohepatitis n=22	Drug-addicted patients with liver cirrhosis n=20
Iodine number by Svrakov, score	1,72±0,02*	2,38±0,06*	2,45±0,05*	2,67±0,02*
Silness-Loe, score	1,21±0,02*	2,16±0,06*	2,18±0,04*	2,35±0,02*
Stallard, score	1,25±0,19*	2,21±0,04*	2,28±0,02*	2,41±0,03*
PMA, %	40,77±0,60*	62,40±0,54*#	62,80±0,89*#	67,32±1,21*#
PBI, score	1,21±0,03*	2,68±0,12*#	2,71±0,11*#	3,08±0,03*#

Note:
* – probability index (p<0,05) compared to comparison group;
# – probability index (p<0,05) compared to group of drug addicts with liver cirrhosis.

in accordance with the principles of the Declaration of Helsinki on Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, and the relevant laws of Ukraine.

RESULTS

All study results, shown in Table 1 indicate that the worst hygiene and periodontal indices were found in tobacco-dependent patients with liver cirrhosis. The results of examination of smokers of all groups of patients with hepatobiliary pathology probably exceeded (p<0,05) the values obtained in the comparison group. (Table I)

As a result of the conducted study it was found out that the examined tobacco-dependent patients with hepatobiliary pathology demonstrated significantly worse values in comparison with patients without somatic pathology. The result of iodine number by Svrakov in tobacco-dependent patients with hepatobiliary pathology was significantly (p<0,05) higher than in the comparison group (1,3 times higher in patients with chronic toxic hepatitis and nonalcoholic steatohepatitis, and 1,4 times higher in patients with liver cirrhosis). Value of Silness-Loe hygiene index in tobacco-dependent persons was significantly (p<0,05) worse than that of the comparison group, in particular, 1,8 times worse in patients with chronic toxic hepatitis and nonalcoholic steatohepatitis, and 1,9 times worse in patients with liver cirrhosis. The values of Stallard index were also significantly higher in smokers with hepatobiliary pathology: 1,7 times higher in patients with chronic toxic hepatitis and nonalcoholic steatohepatitis, and 1,8 times higher in patients with liver cirrhosis (p<0,05).

A similar trend was found during the analysis of the results of PMA index in tobacco-dependent persons: values were significantly (p<0,05) worse than the data in the comparison group (1,4 times worse in patients with chronic toxic hepatitis, 1,5 times worse in patients with nonalcoholic steatohepatitis, and 1,7 times worse in patients with liver cirrhosis). Presence of pronounced inflammatory process in periodontal tissues of tobacco-dependent persons with hepatobiliary system pathology is also evidenced by the values of PBI index, which significantly (p<0,05) exceeded the values of the comparison group (1,4 times higher in patients with chronic toxic hepatitis, 1,7 times higher in patients with nonalcoholic steatohepatitis, and 1,8 times higher in patients with liver cirrhosis).

During examination of oral cavity of drug-addicted patients with hepatobiliary system pathology, unsatisfactory oral hygiene with massive supra- and sub-gingival deposits was observed. Gingivae were significantly hyperemic and swollen, bleeding with slight mechanical irritation. In some addicted patients, namely active users and those who were in the post-abstinence period, xerostomia was observed.

Drug addicts who were in remission for more than 1 year, a decrease in dryness of oral mucosa was reported.

Table II presents the results of index evaluation of the status of periodontal tissues in drug-addicted patients against the background of hepatobiliary system pathology.

The results of the study showed that drug addicts with hepatobiliary pathology had significantly worse values of the investigated indices compared to patients without somatic pathology. In particular, the result of iodine number by Svrakov of the comparison group was significantly different from addicted patients with chronic toxic hepatitis and in patients with nonalcoholic steatohepatitis (by 1,4 times), and 1,5 times different from drug-addicted patients with liver cirrhosis. The value of simplified Silness-Loe hygiene index in drug-addicted patients was significantly worse than that of the comparison group, namely, 1,8 times worse in patients with chronic toxic hepatitis and in patients with nonalcoholic steatohepatitis, and 1,9 times worse in patients with liver cirrhosis. The values of Stallard index were also significantly higher in smokers with hepatobiliary pathology: by 1,8 times in patients with chronic toxic hepatitis and in patients with nonalcoholic steatohepatitis, and by 1,9 times in patients with liver cirrhosis ($p<0,05$).

The results of PMA index in drug-addicted patients show pronounced inflammatory processes in gingivae and are significantly worse than the values of the comparison group (1,5 times worse in patients with chronic toxic hepatitis and in patients with nonalcoholic steatohepatitis, and 1,7 times worse in patients with liver cirrhosis). Intensive development of inflammation in periodontal tissues of drug-addicted patients with hepatobiliary system diseases is also confirmed by the results of PBI index, which are significantly worse than those of the comparison group ($p<0,05$).

When comparing the results of index evaluation in addicted patients with hepatobiliary pathology, it was found that the values of patients with liver cirrhosis are significantly ($p<0,05$) worse than the values of drug-addicted patients with chronic toxic hepatitis and patients with nonalcoholic steatohepatitis (there was no significant difference between the values of the latter).

Research have shown that smoking worsens the course of hepatobiliary pathology and periodontal tissue disease. Also, drug use causes the development of hepatobiliary pathology and the pathology of periodontal tissues

CONCLUSIONS

Study data suggest that periodontal lesions occur much less often in non-addicted patients with inflammatory diseases of periodontium and without concomitant somatic pathologies, and their clinical manifestations are less pronounced than in drug addicts.

Thus, the presence of hepatobiliary system pathology in tobacco- and drug- addicted persons increases the risk of periodontal diseases and contributes to the severity of their manifestations.

Findings suggest that significantly worse oral health status in smokers is caused by not only inadequate care of oral cavity, but to a great extent by the effect of nicotine and its components on the organs and tissues of oral cavity.

With the help of clinical index evaluation, we became certain that addicted patients with hepatobiliary pathology increase the probability of inflammation in the periodontium, and the severity of its course correlates with the degree of development of the underlying disease and the presence of harmful habits.

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ORIGINAL ARTICLE
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COMPLEX PROGRAM OF DIAGNOSTICS AND TREATMENT OF POLYTRAUMATIZED PATIENTS WITH II-III DEGREES OBESITY

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ABSTRACT

The aim: Elaboration of the complex program of diagnostics and treatment of the polytraumatized overweight patients.

Materials and methods: Clinical material was composed of 64 patients with the combined body trauma who suffered from II-III grade obesity.

Results: Main principles of rendering the aid to the polytraumatized overweight patients included the pathophysiological and topographic features of the group. The obtained study results confirmed the credible difference of the traumatic disease progress in the patients with the normal weight and overweight patients which was the basis of our differential complex treatment program.

The treatment tactics also had certain characteristics connected with the obesity. The development of RDS syndrome is a typical stage of the traumatic disease during the blunt thoracal trauma in case of obesity. The programmed and urgent relaparotomy, as a method of the stage treatment in the present group of patients, is the integral component of the blunt abdominal injury in case of obesity.

Conclusions: The study results proved that our complex program of diagnostics and treatment of the polytraumatized patients shall be basic for the patients with II-III grade obesity.

KEY WORDS: polytrauma, obesity, diagnostics, treatment

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INTRODUCTION

It is widely known that the issue of polytrauma is one of the most topical issues in surgery. The patients with the excess weight and different grades of obesity demand special approach among the multitude of the traumatized patients. Thus, as it was shown by some researchers, the overweight injured people suffer more in the course of traumatic disease as compared to the people with the normal weight [1,2,3]. Besides, the references provide only few publications as to the diagnostic characteristics and surgical treatment of the polytraumatized people against the obesity background, and there are practically no clear algorithms as to the diagnostic examination of such patients [4,5].

The latter may be confirmed by the constant growth of the obesity rates, connected with the reduction of the physical activity of population [6]. Specific physiological processes of the obesity cause the most severe injuries, in particular, the skeleton injuries, and high risks of the development of frequently mortal complications. In case of the high BMI, such characteristics of organism play an important role at different stages of medical aid, which preconditions the necessity of specific technical surgical approaches and demand the special surgical nursing [7,8].

The stated facts create the new challenges for the clinician, mainly, in the studying of the diagnostic value of clinical, instrumental, and special methods of examination, elaboration of the differential programs of the complex treatment of patients suffering from the obesity with regard to the characteristics of the traumatic disease and clarifying the impact of the differential complex treatment programs upon the dynamics of major vital indexes (parameters), functional and cosmetic components of the consequences of overweight patients surgical treatment in the format of perspective treatment technology [9,10].

THE AIM

Elaboration of the complex program of diagnostics and treatment of the polytraumatized overweight patients.

MATERIALS AND METHODS

Clinical material was composed of 64 patients with the combined body trauma who suffered from II-III grade obesity. Depending upon the set tasks, the patients were subdivided into two clinical groups – major (34 patients) and approbation (30 patients). Main characteristics of the clinical groups are shown in Table I.

The study consisted of two stages. At stage I the components of the program of diagnostics and treatment of the overweight patients were defined for the patients of the major group, and at the stage II – the outlined principles were applied to the approbation group patients.

Table I. Main characteristics of the clinical groups according to the BMI and sex

	BMI	Age	Men	Women
Major group	37.2 ± 0.3	56.6 ± 1.4	20 (58.8%)	14 (41.2 %)
Approbation group	37.4 ± 0.3	56.2 ± 1.3	17 (56.7%)	14 (43.3 %)

Table II. General characteristics of the closed chest injury

	Ribs fracture			Undrained			Drained		
	n	%	p	n	%	p	n	%	p
Major group	27	79.4	≤ 0.05; χ^2=4.34	11	32.4	>0.05; χ^2=0.04	16	47.1	>0.05; χ^2=2.49
Approbation group	29	96.7		9	30.0		20	66.7	

Table III. Invasive diagnostic methods in case of polytrauma against the obesity background

Clinical group	Abdominal paracentesis		p / χ^2	Diagnostic videolaryngoscopy		p / χ^2
	n	%		n	%	
Major (n=34)	29	85.0	-	6	17.7	-
Approbation (n=30)	30	100.0	≤0.05/ 4.79	5	16.7	>0.05/ 0.01

Within the diagnostics the following methods were applied: general clinical, laboratory and biochemical, instrumental (ultrasonography, craniography, x-radiography of thoracic organs, pelvis, extremities, electrocardiography) and special examination methods (ultrasonography using FAST method, computer tomography, nuclear-magnetic tomography, pathomorphological study of the damaged cells), invasive diagnostic procedures (thoracentesis, abdominal paracentesis (diagnostic peritoneal lavage, videolaryngoscopy), and statistic examination methods.

The scope of medical aid for the traumatized at the pre-hospital stage depended upon the subjective assessment of the patients' general condition by the emergency team. Polytraumatized patients were diagnosed and treated at hospital according to the common and local protocols, which included the collection and analysis of the complaints, medical history and features of the traumatizing factor [1,2,10].

RESULTS

Main principles of rendering the aid to the polytraumatized overweight patients included the pathophysiological and topographic features of the group.

During the diagnostics of the thoracal trauma, apart from the traditional x-radiography which was not always quite informative, for the verification of diagnosis in the patients against the background of II-III grade obesity, the thoracocentesis was applied. Thus, in major group 33 patients (97.1%) were punctured, while in the approbation – 29 (96.7%) which provided for the accurate diagnostics of the pleural and pulmonary complications resulting from the blunt chest injury (($p > 0.05$; χ^2=0.01). General typical characteristics of the blunt chest injury in case of II-III grade obesity are shown in Table II.

Following the stated algorithm of the diagnostics of the blunt chest injury allowed detecting the contusion-laceration of lungs in 100% (($p > 0,001$) and, accordingly, conducting the timely drainage of the pleural cavities.

With regard to the treatment characteristics of the severe thoracic trauma in the patients with II-III grade obesity, and for the purpose of treatment and prevention of the early and late infectious complications through the therapeutic bronchoscopy procedures the tracheostomy is recommended for such patients. Thus, in major clinical group, tracheostomy was performed in 25(73.5 %), approbation group – in 30 (100 %) cases which fully confirmed the necessity of this procedure for the overweight patients ($p \leq 0.05$; χ2=9.24).

Respiratory distress syndrome (RDS) developed in 17 (50.0%) patients of the major clinical group and in 23 (57.7 %) – of the approbation clinical group ($p \leq 0.05$; χ2=4.84).

All 64 (100 %) patients were diagnosed the blunt intra-abdominal injury. For the purpose of diagnostics of the intra-abdominal injuries, apart from ultrasonic diagnosis (using FAST) method, the invasive diagnostic procedures, in particular, abdominal paracentesis (diagnostic peritoneal lavage, videolaryngoscopy and diagnostic videolaryngoscopy were applied to the patients with II-III grades obesity.

As Table III shows it, the invasive methods of the abdominal injuries diagnostics in case of polytrauma in the overweight patients should be predominant among the existing approaches which is confirmed by the calculation results in the approbation group. Positive result of the stated methods was observed in 25 (70.6 %) patients in the major group and in 27 (90.0 %) of the approbation group which became a defining criterion in the indications for the urgent surgical interference ($p > 0.05$; χ^2=2.84).

It should be stated that the trauma of the internals was predominantly composed of the injuries of parenchy-

mal organs, in particular, the spleen lacerations were observed in 14 (41.2 %) of the major and 15 (50.0 %) of the approbation ($p>0.05$; $\chi^2=0.50$) group, and the liver lacerations in 21 (61.8 %)vs24 (80.0 %) correspondingly ($p>0.05$; $\chi^2=2.54$).

The programed or emergency relaparotomy practically in each second patient is the characteristic feature of the course of traumatic disease in patients with II-III grades obesity. Thus, the relaparotomy ratio in the major group was 16 (47.1 %) cases and in the approbation – 19 (63.3 %), which confirmed the correctness of the chosen treatment method ($p >0.05$; $\chi^2=1.70$).

One of the indications for the urgent relaparotomy in case of II-III grade obesity is the development of abdominal compartment syndrome (ACS) diagnosed in 12 (35.3 %) of the major group and in 17 (57.7 %) of the approbation group. The development of ACS in case of obesity caused searching for the optimal treatment method which, for the patients of the present group, was the application of the suspended ileostomy elaborated as based on the patients of the major group, and the grounding of its application was proved on the patients of the approbation group ($p>0.05$; $\chi^2=2.94$).

The next step of the research was the separation of the common skeleton injuries in case of II-III grade obesity. The fractures were typically located in the pelvic bones – 28 (82.3 %) major and 28 (93.3 %) approbation group $p >0.05$; $\chi^2=1.76$), and hip fractures – 14 (41.1%) vs 8 (26.7 %) correspondingly ($p >0.05$; $\chi^2=1.49$).

Fractures of extremities of the other location were more rare, in particular, 12 (35.3 %) in the major and 12 (40.0 %) in the approbation group ($p >0.05$; $\chi^2=1.50$).

The blunt craniocerebral injury was predominantly composed of the light and medium cerebral contusions without the surgical treatment necessity. Thus, in the major group the cerebral contusion was diagnosed in 24 (70.9 %), and in the approbation in 20 (66.6 %) cases ($p>0.05$; $\chi^2=0.11$).

Median hospital treatment duration in case of polytrauma against the background of II-III grade obesity in the major group was 31.0 ± 1.6, and in the approbation – 30.7 ± 1.5 bed-days ($p>0.05$). Mortality in the major group was 5.9% (n – 2) in the approbation – 3.3 % (n – 1); $p>0.05$; $\chi^2=0.23$.

DISCUSSION

The obtained study results confirmed the credible difference of the traumatic disease progress in the patients with the normal weight and overweight patients which was the basis of our differential complex treatment program. Noteworthy that our results were correlated with the data of the other authors [8,9] who stated that the diagnostic accuracy of some visualization examination methods of the patients with the polytrauma is reduced in direct ratio to the grade of obesity. The higher obesity grade the smaller percent of the instrumental diagnostic approaches reliability in such patients. This causes the necessity of more frequent implementation of the invasive methods, which, on the one hand improved the injuries verification, and on the other – provoked the risks of complications connected both with their implementation and intervention into the body cavities [1,6].

The treatment tactics also had certain characteristics connected with the obesity. The development of RDS syndrome is a typical stage of the traumatic disease during the blunt thoracic trauma in case of obesity, in the treatment of which, apart from the drug treatment, the therapeutic bronchoscopy is predominant, which caused the necessity of tracheotomy on 2-3 day from the moment of the artificial pulmonary ventilation.

The programmed and urgent relaparotomy, as a method of the stage treatment of the traumatic disease in the present group of patients, is the integral component of the blunt abdominal injury in case of obesity. The said approaches proved their credibility in the treatment of ACS through the application of the suspended ileostomy.

CONCLUSIONS

The study results proved that our complex program of diagnostics and treatment of the polytraumatized patients shall be basic for the patients with II-III grade obesity.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

THE ROLE OF CYTOCIN IMBALANCE IN THE DEVELOPMENT OF MAN INFERTILITY

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ABSTRACT

The aim: Study of the clinical and pathogenetic role of IL-17, IL-35 and their correlation in the development of infertility in men with chronic ureoprostatitis.

Materials and methods: 82 male aged 20 to 40 were examined. The patients were divided into three groups: the first – 10 practically healthy men, in which the levels of IL-17 and IL-35 in semen were taken as normal; second – 33 infertile men with chronic urethroprostatitis; third – 39 fertile men with chronic urethroprostatitis.
In addition to conventional clinical and laboratory (clinical blood and urine tests) studies, all men in semen were tested for IL-17 and IL-35 cytokine levels.

Results: The levels of the studied cytokines were found to have opposite tendencies to shifts in the semen of men with chronic urethroprostatitis. The level of IL-17 in sperm increases and the level of IL-35 decreases. The IL-17/IL-35 index increases more strongly in men with impaired fertility.

Conclusions: Increas IL-17 concentrations, decreas IL-35 levels and increas their correlation may be an indicator of infertility in men with chronic ureoprostatitis. Determining the IL-17 / IL-35 correlation in general clinical practice will allow to single out a group of men with a high likelihood of developing infertility for follow-up and treatment by a doctor of family medicine.

KEYWORDS: urethroprostatitis, cytokines, male infertility

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INTRODUCTION

The interest in reproductive health is caused by the emergence of numerous reports of a decrease in the quantitative and qualitative characteristics of semen, as well as an increase in cases of pathology of the male sexual sphere in the late XX – early XXI centuries, accompanied by the development of infertility [1,2]. The majority of patients cannot determine the cause of fertility decline, so the search for new diagnostic approaches remains relevant. The fine mechanisms of immune regulation of gametoid formation in men are being studied. It has been established that certain combinations of cytokine levels in the gonads create the optimal microenvironment for the maturation of generative cells [3]. Therefore, the violation of the concentration of certain cytokines in the gonads leads to a decrease in fertile function of men, as well as the development of autoimmune diseases [4].

During the course of the infectious process, the activation of the corresponding receptors and signal transduction pathways, accompanied by the release of biologically active substances such as proinflammatory cytokines [5]. Secreted proinflammatory cytokines are mediators of the host response to infection [6]. It is well known that the lymphocytic population of CD4 + T helper – (Th) cells can be distributed, in addition to T regulatory (Treg), Th9, Th17, Th22 and follicular (Tfh), also into two subpopulations of Th1 and Th2 on the basis of cytokines produced by them [7,8,9]. Th1 cells mainly secrete IL-2, IFN-γ, and IL-12 interleukin, whereas Th2 cells secrete IL-4, IL-5, and IL-10. Functionally, Th1 cells mainly promote cell-mediated immunity and eliminate intracellular pathogens; Th2 cells are responsible for humoral immunity for protection against extracellular pathogens. The balance between these multifaceted cytokines, in particular between IL-12 and IL-4, determines the nature of the immune response [8]. IL-17 is a pro-inflammatory cytokine produced by the recently described Th17 cells, which play a critical role in the immune response against extracellular bacteria, as well as in the pathogenesis of some autoimmune disorders [9]. IL-6 and TGF-β are responsible for the generation of Th17 cells. At the same time, IL-17 products are supported by IL-1b, IL-15, IL-23, or TNF-α. A number of researchers have obtained results confirming the possibility of stimulation of IL-17 by a factor inhibiting macrophage migration (MIF) [10,11,12,13]. The role of IL-17 in the development of male infertility is poorly understood. IL-35 is part of the interleukin family that includes IL-12, IL-23, IL-27, and IL-35. IL-35 has an immunosuppressive effect on Th17 mediated through stimulation of Treg cells [14,15,16,17]. Under the influence of IL-35, CD4 + CD25 + T cells produce IL-10, and CD4 + CD25- T cells produce gamma interferon. In addition, IL-35 inhibits Th17 cell differentiation. Thus, IL-35 is an anti-inflammatory cytokine that inhibits the immune response [18,19,20].

Changes in the proportion of cytokines (eg, IL-10: IL-12) are thought to be key in inhibiting immune defenses in the

Table I. IL-17 and IL-35 levels in semen of men with chronic urethroprostatitis (Me±[Q25; Q75])

Indicator of immune status	Norm (control group men)	Group I (infertile men)	Group II (fertile men)	P
IL-17, PG/ml	14,2 [11,4;17,4]	25,3 [19,6;29,9] **	18,6 [17,5;22,2] *	<0,01
IL-35, PG/ml	52,3 [28,6;71,4]	16,2 [11,9;24,4] ***	38,9 [26,5;50,2] *	<0,01
IL17/IL35	0,27[0,21;0,39]	1,56 [0,71;2,43] ***	0,47 [0,35;0,63] *	<0,01

Note: the reliability of the difference of indicators in comparison with the norm P: * - <0,05, ** - <0,01, *** - <0,001; P is the significance of the difference between the groups.

Table II. Gradations of IL-17 and IL-35 content in semen of men with chronic urethroprostatitis

Indicator of immune status	Fertility	Grades of the indicator				
IL-17, PG/ml		14-17	18-21	22-25	26-29	29 and more
	fertile	18/46,1	10/25,6	7/18,0	14/10,3	0
	infertile	3/9,1	3/9,1	11/33,3	7/21,1	9/27,3
	norm	14,2±1,5				
IL-35, PG/ml		11-21	22-32	33-43	44-54	55 and more
	fertile	4/10,2	11/28,2	1/2,6	5/12,8	18/46,2
	infertile	12/36,4	11/33,3	6/18,2	4/12,1	0
	norm	52,3±10,8				
IL17/IL35		0,2-0,8	0,9-1,5	1,6-2,2	2,3-2,9	3,0 and more
	fertile	21/53,8	8/20,5	10/30,8	0	0
	infertile	0	16/48,5	12/36,4	4/12,1	1/3,0
	norm	0,27±0,08				

Note: in the numerator - the absolute number of patients, in the denominator - % to the number of patients examined

lower parts of the genital tract, inhibiting cell-mediated immune responses and immunosuppression [21,22,23].

Based on the previously proven role of IL-17 as a major mediator of the development of chronic inflammation and autoimmune damage, and IL-35 as a cytokine, which in turn inhibits the inflammatory process and the development of autosensitization, we were tasked with investigating the IL-17 / IL-35 ratio in sperm men with chronic urethroprostatitis depending on fertile function.

THE AIM

Study of the clinical and pathogenetic role of IL-17, IL-35 and their correlation in the development of infertility in men with chronic ureoprostatitis.

MATERIALS AND METHODS

There were 82 men aged 20 to 40 under our supervision. All patients gave informed written consent to participate in the study, which was approved by the Bioethics Committee of Ternopil NMU Ministry of Health of Ukraine. The researches were carried out in accordance with ethical norms and moral and legal requirements of the order of the Ministry of Health of Ukraine No. 281 of 01.11.2000. The patients were divided into three groups: the first group consisted of 10 healthy men whose IL-17 and IL-35 levels in semen were normal; the second – 33 infertile men with chronic urethroprostatitis; third – 39 fertile men with chronic urethroprostatitis. In addition to conventional clinical and laboratory (clinical blood and urine) studies, all men in semen were tested for IL-17 and IL-35 cytokine levels. In patients with chronic urethroprostatitis, the presence of sexually transmitted infections has been confirmed by the polymerase chain reaction method. The duration of the disease ranged from 1 to 10 years.

All patients were examined clinically, including examination and palpation of the penis, scrotum, and prostate, and a history was carefully collected. All patients donated semen for analysis. On the eve of the assay, no ejaculation had to occur within 4-5 days. The semen was analyzed half an hour after the ejaculation and according to the WHO instruction [24]. They took into account the sperm concentration, the pH of the semen, the leukocyte content. The study did not include patients with leukospermia. The semen was centrifuged before analysis at 1,700 rpm to separate the sperm from the family plasma. IL-17 and IL-35 cytokine concentrations in seminal plasma [25] were determined by the enzyme immunoassay using a STAT-FAX-303 PLUS analyzer (USA) at 492 nm and the Panomics Quantitative Assays test system by «Affymetrix» (USA). Control values for the concentrations of the above

cytokines were obtained in a study of 10 fertile men without clinical signs of disease, similar in composition to age.

Statistical analysis of the obtained results was performed in the environment of the licensed statistical package of the MedStat program [26]. At the same time, the Shapiro-Wilk test was used to test the indicators for normal distribution. Since all the data belonged to a distribution other than normal, they are given in the form Me [Q25; Q75]. The median was calculated, 25% quartile, 75% quartile. The criterion $\chi 2$, the two-sided critical region, was used to compare the indicators. To compare the mean values of the trait for two independent samples, Fisher's angular transformation method was used, taking into account the Yeats' correction. Significant was considered the difference at $p<0.01$. Kendall's paired correlation coefficient – τ was calculated to analyze the presence and strength of the link.

RESULTS AND DISCUSSION

Studies have shown that the majority of men with chronic prostatitis complicated with infertility have detected shifts in the concentration of IL-17 and IL-35 in semen.

An immunological study showed (Table I) that the examined patients with infertility showed a significant decrease in IL-35 family plasma (16.2 ± 3.7 pg / ml, $p<0.001$) on the background of a significant increase in IL-17 concentration (25.3 ± 2.4 pg / ml, $p<0.01$). In fertile men, these indicators also had a significant difference from the normative indicators, but the multiplicity of the difference was smaller. Thus, for infertile men, the multiplicity of difference for IL-17 and IL-35 was respectively 1.8 and 3.2. For fertile men, the multiplicity of difference was 1.2 and 1.4, respectively. The study of the IL-17 / IL-35 index showed its increase in men with chronic uretroprostatitis, complicated by infertility, to 1.56 ± 0.51, $p<0.001$, which is 3.3 times higher than in men with chronic urethroprostatitis with preserved fertility.

A study of the gradations of IL-17 and IL-35 cytokine levels in the semen of men with chronic urethroprostatitis depending on fertile function showed that in patients with preserved fertility 46.1% for IL-17 and 59.0% for IL- 35 their values did not go beyond the normative ones (Table II).

In infertile men, cytokines IL-17 and IL-35 in the semen of men with chronic urethroprostatitis remained within the normative range of only 9.1% and 12.1%, respectively. As for the immunological index IL-17 / IL-35, in infertile men with chronic urethroprostatitis in no patient this ratio did not acquire normative values. At the same time in fertile men with chronic urethroprostatitis, this ratio was within the normative values in almost half of the patients. Therefore, it was considered appropriate to study the IL-17 / IL-35 ratio, which may be informative enough to determine fertile function in men with both chronic urethral prostatitis and other urogenital tract lesions.

Therefore, it was found that the levels of cytokines under study had opposite tendencies to shifts in semen of men with chronic urethroprostatitis. The level of IL-17 in sperm increases and the level of IL-35 decreases. The IL-17 / IL-35 index is statistically significantly higher in men with impaired fertility.

Determination of IL-17 and IL-35 levels may be a biomarker for chronic inflammation of the urogenital tract in men in sperm serum. During the development of infertility in the sperm, the concentration of IL-17 increases and the concentration of IL-35 sharply decreases. Disruption of cytokinogenesis in men with chronic urethroprostatitis leads to the development of infertility. The index of IL-17/IL-35 may serve as a marker of infertility.

CONCLUSIONS

1. The biomarker of chronic inflammation of the urogenital tract in men with semen may be to determine the levels of IL-17 and IL-35.
2. During the development of infertility in sperm, the concentration of IL-17 increases and the concentration of IL-35 sharply decreases.
3. Impairment of cytokinogenesis in men with chronic urethroprostatitis is associated with the development of infertility. The index of IL-17 / IL-35 may serve as a marker of infertility.
4. Determining the IL-17 / IL-35 correlation in general clinical practice will allow to single out a group of men with a high likelihood of developing infertility for follow-up and treatment by a doctor of family medicine.

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ORIGINAL ARTICLE
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BONE MINERAL DENSITY AND TRABECULAR BONE SCORE IN POSTMENOPAUSAL WOMEN WITH KNEE OSTEOARTHRITIS AND OBESITY

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ABSTRACT

The aim of the study was to investigate the relationship between bone mineral density (BMD) of lumbar spine, femoral neck, trabecular bone score (TBS) and body mass index (BMI), in postmenopausal women with knee osteoarthritis (OA).

Materials and methods: The study group comprised 359 postmenopausal women aged 50-89 years. They were divided into 2 groups: I group – 117 postmenopausal women with symptomatic knee OA and II group –242 women with a normal functional activity of knee joints. Analysis of data was performed taking into account their BMD of lumbar spine (L1-L4) and femoral neck, measured by the Dual-energy X-ray absorptiometry (DXA) Hologic (Discovery WI, USA, 2016). TBS of L1-L4 was detected by TBS insight® software (MedImaps, Pessac, France), and BMI classified by World Health Organization (WHO).

Results: In postmenopausal women with obesity prevalence of symptomatic knee OA was detected in 41.1% of cases. However, in women with normal BMI knee OA was revealed in 29.0% of women. The highest level of knee OA in obese women aged 70-79 years – 45.8%. According to a chi-squared ($\chi 2$) test, a significantly higher level of BMI was detected in postmenopausal women with OA ($\chi 2=5.05$, $p=0.02$).

Conclusion: Women with a symptomatic OA had a significantly higher BMD of lumbar spine compared with women who had a normal functional activity of knee. Significant negative correlation were detected between TBS and BMI, and significant positive correlations between lumbar spine BMD and BMI.

KEYWORDS: bone mineral density (BMD), osteoporosis (OP), obesity, postmenopausal women, knee osteoarthritis (OA)

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INTRODUCTION

Musculoskeletal disorders (MSDs) are the leading chronic condition that results in deterioration the health level of workforces among European population. Data released by the Ministry of Health of Ukraine suggest that MSDs widely spread in our country among the working age group. The total amount of people suffers from MSDs in Ukraine are up to 4 000,000, and near 2 000,000 among them are aged 35 – 60 years old [1]. The most prevalent MSDs are osteoarthritis (OA) and osteoporosis (OP) the number of which increases with age.

However, in a case obesity adjunction, the course of any disease worsens. The amount of people with overweight and obesity has highly increased and has nearly tripled since the last decades of previous century [2,3]. Nowadays, all over the world, are being conducted an important discussion about OA and OP that is focused on whether being overweight and obese may have a detrimental or protective effect on skeletal health [4]. On the one hand, some studies indicates inverse relationship between OA and OP, as opposite to this the increased bone mineral density (BMD) in patients with OA does not resulting in reducing risk of osteoporotic fractures [5]. At the same time some studies concludes that obesity has protective role on bone health, while others shows its detrimental effects [6].

THE AIM

The aim of the study was to investigate the relationship between BMD of lumbar spine, femoral neck, TBS and body mass index (BMI), in postmenopausal women with knee OA and without.

MATERIALS AND METHODS

The study was performed in D. F. Chebotarev Institute of gerontology, NAMS of Ukraine in accordance with the cooperation agreement between D. F. Chebotarev Institute of gerontology, NAMS of Ukraine and P. L. Shupyk National Medical Academy of Postgraduate Education (NMAPE) from 15 April 2019. The study was approved by the Ethical Committee of NMAPE (05.11.2018, Protocol № 10). All the participants had signed a voluntary informed consent form for participation in research, being the subjects to the respective diagnostic examination procedures.

The study included 359 postmenopausal females aged 50-89 years old, that were divided into four groups by age decades: 87 women aged 50-59 yrs, 162 women aged 60-69 yrs, 88 – aged 70-79 yrs, and 22 women over 80 years old.

The study groups consisted of 117 females with a symptomatic knee OA (I group) and 242 patients without osteoarthritis

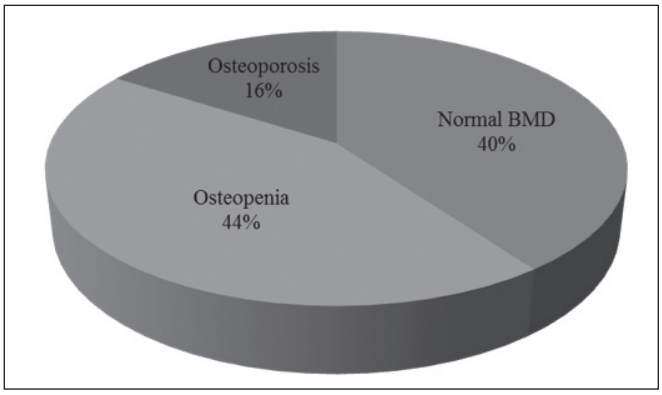

Fig. 1. Distribution of patients according BMD

(II group). Diagnosis of a symptomatic OA was established according to the American College of Rheumatology (ACR) Clinical classification criteria for knee and hip OA (1986). According to these criteria, the presence of knee pain along with at least three of the following six items may be used to diagnose the knee OA: age after 50 years old, morning stiffness of less than 30 minutes, crepitus of knee motion, bone tenderness, bone enlargement, no palpable warmth [7,8]. Lower prevalence estimates for a symptomatic rather than a radiographic OA in the general population reflect the fact that a radiographic OA is not always accompanied by a clinical disease [9].

The BMD of lumbar spine L_1-L_4 and femoral neck was measured by the Dual-energy X-ray absorptiometry (DXA) Hologic (Discovery WI, USA, 2016). TBS of L_1-L_4 was detected by TBS insight® software (MedImaps, Pessac, France), which is installed on DXA. Diagnosis osteoporosis was established according to the WHO group described criteria. OP is defined as spinal (L_1-L_4) or hip (femoral neck) BMD T-score is equal to or below 2.5 standard deviation (SD) of the young-adult reference mean BMD. Osteopenia is defined as a BMD T-score between- 1.0 and -2.5 SD, and normal BMD – within 1.0 SD.

BMI was computed by the ratio of body weight (kilograms) and height^2 (meters), expressed in kg/m^2 (WHO, 1998). Diagnosis of obesity was established when BMI was over 30 kg/m^2.

The results are presented in the following manner: Mean values (M) ± SD. For data calculation we used: regression, correlation analysis and chi-squared test. A result were considered significant if "p" values were lower than 0.05 ($p<0.05$). "Statistika 10.0" (StatSoft, Inc. ©) was used for data processing purposes.

RESULTS

In females with knee OA were detected significantly higher weight and BMI compared with females without OA (table I). However, there were observed no differences between age and height across all groups.

In this study, no significant differences were found either for group I or II while analyzing the BMD of femoral necks ($p=0.07$ and $p=0.33$ respectively) and TBS ($p=0.06$). However, the women with OA had a significantly higher BMD of lumbar spine compared with woman who had a normal functional activity of knee ($p=0.000068$) (Table II).

With DXA-based BMD measurement of observed individuals, OP was detected in 54 women (16% of patients). In 158 females was osteopenia and in 144 was normal BMD (Fig.1).

In both groups there were observed no significant correlation between BMD of L1–L4, hip and age (Fig. 2) At the same time significant negative correlation were detected between TBS and BMI, therefore in a patients with higher BMI, TBS decreases (Fig.3). Correlation and regression analyses between BMI and BMD lumbar spine also shows significant positive relations, what means that at the same time with increasing of BMI increases BMD of lumbar spine (Fig.4).

In the obese subjects, OA was detected in 44 postmenopausal women (41.1%), while a normal functional activity of knee – in 63 women (58.9%). However, out of 73 women with a normal BMI, 29.0% of cases had a symptomatic OA.

In 42.9% of women with obesity aged 50-59 years was detected knee OA comparing with 57.1% of women without OA. In the group of 60-69 years with obesity, there were

Table I. Anthropometric characteristic of examined women

Parameters	Group I	Group II	F	P
Age, years	65.4 ± 8.41	65.8 ± 7.80	0.24	0.62
Weight, kg	75.3 ± 15.30	72.1 ± 12.68	4.39	0.04
Height, cm	161.9 ± 6.38	162.2 ± 6.90	0.20	0.65
BMI, kg/m^2	28.8 ± 5.68	27.4 ± 4.87	6.45	0.01

Note. Group I – postmenopausal women with knee OA, Group II – postmenopausal women without OA.

Table II. Bone mineral density and trabecular bone score in examined patients

Parameters	Group I	Group II	F	p
TBS	1.24 ± 0.11	1.22 ± 0.10	3.32	0.06
BMD of lumbar spine	0.90 ± 0.16	0.83 ± 0.01	16.3	0.00
BMD of right femoral neck	0.67 ± 0.12	0.65 ± 0.11	3.12	0.07
BMD of left femoral neck	0.66 ± 0.12	0.65 ± 0.10	0.91	0.33

Note. Group I – postmenopausal women with knee OA, Group II – postmenopausal women without OA.

detected 39.3% of patients with symptomatic OA comparing with 60.7% of females with a normal functional activity of knee. In the group of obese women of 70-79 years, knee OA was revealed in 45.8% and normal functional activity of knee in 54.2% of participants. In the oldest group of subjects over 80 years, the OA was in 33.3% comparing with 66.7% of woman with a normal functional activity of knee and obesity.

Among women with a normal BMI aged 50-59 years old knee OA was detected in 31.8% of cases comparing with 68.2% of those with a normal functional activity of knee joint. In group of 60-69 years, OA was detected in 27.2% of women comparing with 72.6% of those without OA. In the group of 70-79 years, there were 25.0% cases with symptomatic OA and normal functional activity of knee joint was revealed in 75.0% of women. In the oldest group of subjects over 80 years, the distribution was 43.8% of patients knee OA comparing with 56.2% of women with a normal functional activity of knee joints.

According to chi-squared ($\chi 2$) test, a significantly higher level of BMI, or more precisely, obesity, was detected in postmenopausal women with knee OA ($\chi 2=5.05$, $p=0.02$). In the first group, there were 44 women with obesity and 73 with normal body weight. In the second group – 63 subjects had obesity and 179 – a normal BMI. In the group of 50-59 year-old women, the values were $\chi 2=0.86$, $p=0.68$, in the group of 60-69 year-olds the values were: $\chi 2=2.42$, $p=0.12$, in the group of 70-79 year-old the values were: $\chi 2=3.56$, $p=0.05$, and in the group of women over 80 years the values were $\chi=20.20$, $p=0.65$.

DISCUSSION

According to the data of the WHO, every one in 4 adults in the world has an overweight and every one in 11 – obesity. Such a high rate of body weight-related problems is alarming due to the fact that an overweight or obesity itself is a risk factor for dozens of diseases. It can cause premature disability and death by increasing the risk of cardiometabolic diseases, OA, dementia, depression and some types of cancers [3].

Nowadays, all over the world, are being conducted an important discussion about obesity, OA and osteoporosis that is focused on whether being overweight and obese may have a detrimental or protective effect on skeletal health [4]. Historically, obesity has been linked to bone health as a protective factor consequently, some researchers suggest that obesity has protective role on bone health, while others have revealed its detrimental effects. Weight loss in miscellaneous populations including pre- and postmenopausal women leads to a loss of total body BMD up to 2.5% as well as variable losses at regional bone sites 1%–13%. According to the literature data, in premenopausal women greater weight loss (average 14%) during a relatively short period of time (3–4 months) results in significant bone weakening however a modest weight loss over a longer period of time (6 months) results in fewer or no bone loss [6].

The mechanism behind obesity starts with a similar origin of osteoblasts and adipocytes, both being precursors of a mesenchymal stem cell (MSC). Aging alone alters the MSC in the bone marrow by promoting adipogenesis and reducing osteoblastogenesis [10]. Visceral abdominal fat is the most metabolically active and may be associated with a poor quality of bone tissue and lower BMD. The latter one is also associated with a higher frequency of falls, reducing up to 40% fat free mass composed mostly of skeletal muscle in the elderly [11] and lower bioavailability of vitamin D accumulated and stored in the fat tissue [12].

Besides, obesity affects the bone metabolism through multiple pathways, including an alteration of bone-regulating hormones, androgens-to-estrogens conversion in adipose tissue, lower serum levels of Sex Hormone Binding Globulin (SHBG), increased serum leptin levels, increased insulin growth factor production and hyperinsulinemia, inflammation, oxidative stress, endocannabinoid system [6, 10].

All of these mechanisms could be further enhanced by aging that is another topical issue nowadays [10]. Together with an increasing rate of global aging, the number of people with OA is noticeably rising. OA characterized by the degradation of cartilage in joints and leads to bones rubbing together and results in stiffness, pain, and impaired movement (WHO) [13]. It usually results in decreased mobility and obesity is a major comorbidity in such patients [14]. Worldwide OA is estimated to affect 15% of people of different ages [13]. The relationship between OA and OP is complex and controversial [5].

Epidemiological studies suggest that in the same patient OA and OP rarely occur together. In addition, cross-sectional studies have indicated that OA is associated with an increase in BMD leading to a common assumption that an increased BMD is protective against OA progression [15].

In the Korean National Health and Nutrition Examination Surveys, there were observed 5793 persons with OA. Their lumbar spine BMD was significantly higher than the one of subjects with knee OA. The findings prompted the conclusion about an inverse relationship between OP and the presence of knee OA; however, there is a non-linear and site-specific association between OP and the severity of knee OA [16]. In the Rotterdam study, patients with knee OA had a higher BMD, however the incident fracture risk was increased as compared with those without knee OA [5].

Undoubtedly, there is a certain correlation between BMD and severity of OA. There was reported an increased BMD at all sites in subjects with a moderate–severe OA of either hip, increased BMD at femoral neck and lumbar spine in subjects with a milder hip OA [17].

The Surveys of Osteoarthritis Research Society International (OARSI) consider obesity to be a strong risk factor for knee OA that may also increase the rates of hip, hand and spinal OA, and the number of these painful conditions, together with their associated disability and loss of function, will continue to increase [18].

The Framingham study described an example of decreasing the risk of developing knee OA by 50% in losing weight females, on average 11 pounds (1 pound equals 453.59 g). The Clifford survey conclude the possibility of development knee OA increases in 1.36 times with every two units of putting on weight (approximately equal to 5 kg) [19].

Recent data from the Intensive Diet and Exercise for Arthritis study suggests that non-mechanical risk factors also play a part in OA development. Weight loss in obese subjects with concomitant knee OA may have anti-inflammatory, as well

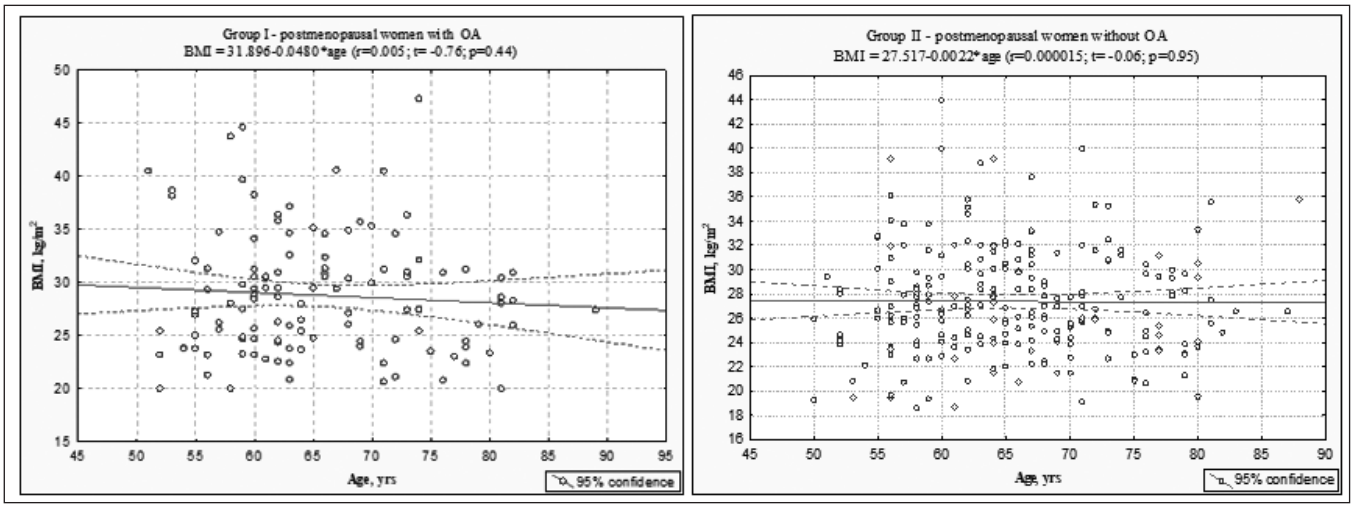

Fig.2. Correlation and regression analyses of relations between BMI and age in postmenopausal women with and without OA.

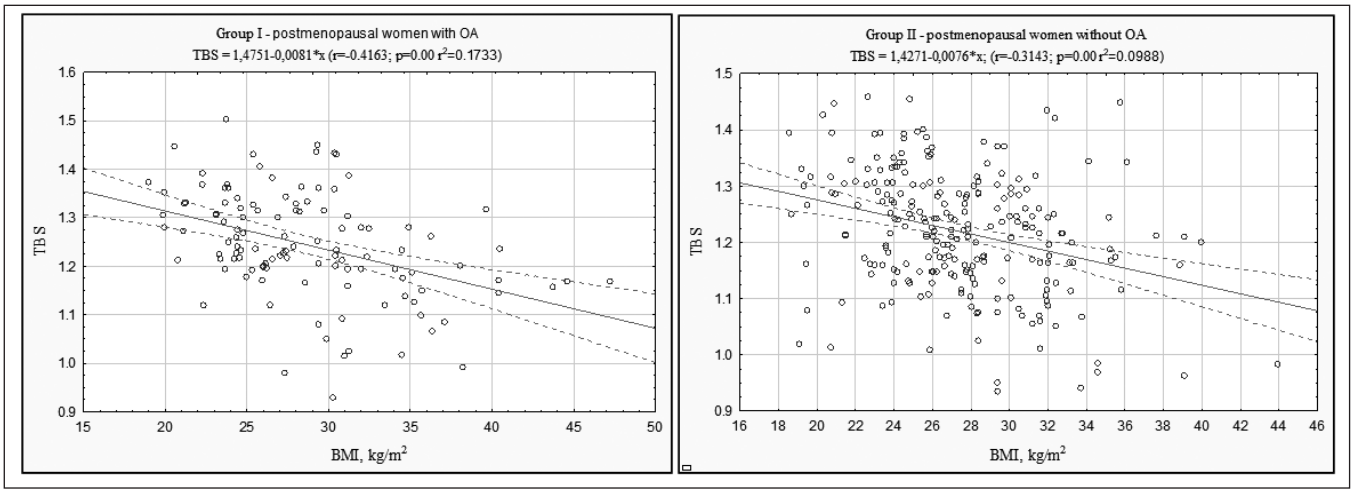

Fig.3. Correlation and regression analyses of relations between BMI and TBS in postmenopausal women with and without OA:

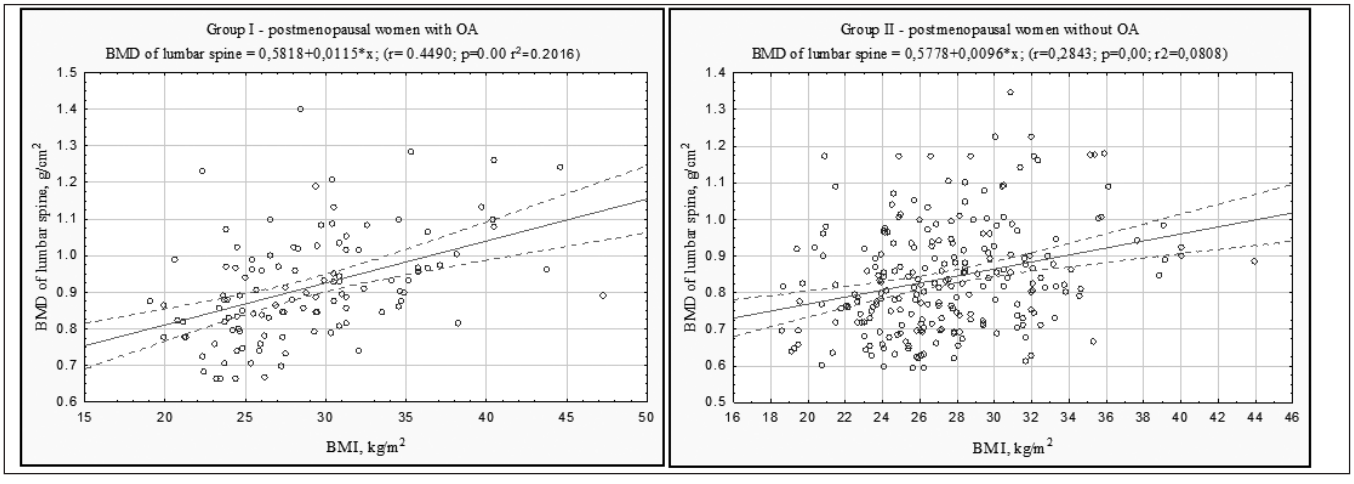

Fig.4. Correlation and regression analyses of relations between BMI and BMD lumbar spine in postmenopausal women with and without OA:

as biomechanical, benefits, that evidenced by reduced IL-6 levels. Furthermore, beside an association between obesity and OA in weight-bearing joints such as the knee and hip, obesity is also associated with the development of OA in non–weight-bearing joints, such as those in the hand [20].

This year, a group of Italian scientists was studied TBS in 352 postmenopausal women with obesity. As a result, BMI was found to be negatively related to TBS and positively to the lumbar spine BMD [21, 22]. A higher BMD has also been reported in association with OA of the spine [9].

CONCLUSION

The rates of obesity in patients with knee OA, is significantly higher compared to persons with a normal functional activity of knee. Postmenopausal women with knee OA had a significantly higher BMD of lumbar spine compared with women who had a normal functional activity of knee joints. Significant negative correlation were detected between TBS and BMI, and positive correlations between lumbar spine BMD and BMI.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

THE ROLE OF PHYSICAL EDUCATION IN IMPROVING THE HEALTH STATUS OF STUDENTS OF SPECIAL MEDICAL GROUPS

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ABSTRACT

The aim is to carry out the analysis of the disease incidence of students and to scientifically substantiate the directions of improving health and fitness activity in the physical education of the students of special medical groups.

Materials and methods: The study was conducted at Zhytomyr National Agroecological University in 1992-2019. The main pedagogical experiment involved 681 students (209 males and 472 females, aged 17-22). The research methods included theoretical methods (the analysis and synthesis of literary sources, the analysis of curricula, the assessment of the quality of classes with the students of special medical groups, the study and analysis of the medical cards of students); empirical methods (pedagogical observations, the methods of mathematical statistics).

Results: The study determined that more than 57.6 % students of higher educational establishments of Ukraine had an unsatisfactory level of physical fitness, significant physical disabilities, diseases; from 10.9 to 20.5 % students had an insufficient level of physical fitness and significant health disorders; from 8.1 to 17.4 % students belonged to a special medical group; from 0.4 to 1.2 % students were exempt from sports and had chronic diseases and disabilities.

Conclusions: The quality of the physical education process in special medical groups depends on the interconnection of all the components that affect its efficiency, including an outlook, motives, interests, the attitude of students to physical education and health-improving activities; the students' desire to overcome their health disabilities; individual characteristics of students; the means, forms, methods, and principles of physical self-improvement; material and technical support of educational and health and fitness events.

KEY WORDS: physical education, special medical group, disease incidence, physical fitness, students

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INTRODUCTION

The main task of physical education classes in special medical groups is the elimination of residual after diseases, defects of the musculoskeletal system, functional abnormalities, deficiencies of physical development, and also the improvement of the level of physical fitness and necessary skills and abilities to use physical exercises for the health rehabilitation [1, 2, 3]. The students who belong to a special medical group train according to special programs that are developed by a teacher, and sometimes with the help of a doctor, taking into account the forms of diseases and the nature of physical abnormalities of particular students. Occasionally, the classes may be conducted according to the separate sections of the physical education program in a special training department, but with significantly reduced standards and extended deadlines. In some cases, therapeutic or corrective gymnastics classes may be held [4, 5, 6].

At the same time, the system of the physical education of students who have chronic diseases, health problems, congenital anomalies and the low levels of physical development and physical fitness, do not completely solves the problem of the restoration of working capacity and ensuring the normal functioning [7, 8, 9, 10, 11]. One of the causes of the disease incidence and health problems of students of Ukraine is the unsatisfactory condition of the physical education system at schools and higher educational establishments and its obsolescence [12, 13, 14, 15, 16]. If one summarizes the essence of the shortcomings in the system of physical education of the students of special medical groups, suggestions, and requirements of teachers regarding its reformation, it becomes clear that one can find the necessary ways to solve this problem by modernizing the system of physical education. The modernization of the system of physical education of the students of higher educational establishments of Ukraine, which the Ministry of Education and Science of Ukraine has been conducted recently, leads to the fact that physical education as a discipline loses its ability to solve simultaneously educational, pedagogical, and health-promoting problems that can provide a balanced pedagogical impact on

Table I. The indicators of medical examination of the applicants and first-year students of Zhytomyr National Agroecological University in 1992-2019 (%)

The year of examination	The number of people	Medical groups			With special needs
		The main group	The group with reduced activity	The special group	
1992	896	69.2	20.5	9.8	0.5
1993	999	72.1	18.9	8.2	0.8
1994	516	73.4	17.1	8.7	0.8
1995	491	71.1	19.6	8.1	1.2
1996	817	73.3	14.4	11.4	0.9
1997	754	76.0	13.0	10.3	0.7
1998	709	68.9	17.3	12.7	1.1
1999	650	69.9	16.6	13.1	0.4
2000	773	70.7	15.4	13.4	0.5
2001	931	69.4	12.8	17.4	0.4
2002	998	73.9	10.9	14.7	0.5
2003*	1025	87.7	–	11.4	0.9
2004*	1118	88.2	–	10.7	1.1
2005*	1180	89.2	–	9.2	1.6
2006*	1149	86.7	–	11.1	2.2
2007*	1161	85.4	–	14.0	0.5
2008*	1018	80.1	–	18.3	1.6
2009*	1093	85.6	–	13.6	0.8
2010*	1133	89.7	–	9.7	0.6
2011*	875	87.5	–	11.9	0.6
2012*	727	85.9	–	13.6	0.5
2013*	658	76.7	–	22.9	0.4
2014*	677	74.3	–	25.5	0.2
2015*	567	78.6	–	21.2	0.2
2016*	548	77.2	–	22.7	0.1
2017*	698	69.0	–	30.4	0.6
2018*	762	69.5	–	30.1	0.4
2019*	724	55.1	–	44.1	0.8

Note. From 2003 to 2019, the table presents data of the first-year students entered the university

a student, according to the requirements and possibilities of forming a creative, intellectual, morally stable and physically advanced personality [3, 11, 17, 18].

The physical education system existing at higher educational establishments of Ukraine cannot completely overcome the students' motor activity deficit; ensure efficient recovery, preservation, and improvement of the health of young students. The improvement of the efficiency of health promotion of the students of special medical groups can be achieved by reforming the methodical points of the physical education system, introducing a four-level model of health and fitness activities that includes a target component (the system of goals of the development of physical education and the formation of motives and interests in the activity); a content component (modern educational and methodical support); a procedural component (methods, forms, and means of training); a diagnostic and reflexive component (the control and evaluation of training efficiency).

THE AIM

The aim of the study is to carry out the analysis of the students' disease incidence and to substantiate scientifically the directions of improving health and fitness activity in the physical education of the students of special medical groups.

The objectives:

1) to conduct a systematic analysis of the physical condition and disease incidence of the applicants and students of higher educational establishments of Ukraine during a long historical stage;
2) to solve scientific and methodical problems of health and fitness activity in physical education, aimed at the elimination of residual after diseases, defects of the musculoskeletal system, functional abnormalities, and a low level of the physical development of the students of special medical groups.

Table II. The structure of the disease incidence of the students of a special medical group (n=681, 209 males, 472 females; % of the total number of diseases)

The classes of diseases	Gender	The year				Totally
		1st	2nd	3rd	4th	
The diseases of the blood, blood-forming organs, and circulatory system	males	45.5	43.7	28.9	8.3	37.2
	females	34.1	35.9	32.8	20.0	33.1
The diseases of the musculoskeletal system	males	10.6	18.3	14.5	16.7	14.5
	females	17.4	11.6	8.6	11.1	12.5
The diseases of the urogenital and endocrine systems	males	12.1	10.9	14.5	–	12.1
	females	11.6	9.2	12.9	22.3	12.1
The diseases of vision	males	4.5	3.6	6.6	25.0	6.3
	females	8.7	10.4	4.3	4.4	7.8
The diseases of the gastrointestinal tract	males	4.6	1.8	9.2	8.3	5.8
	females	2.9	7.5	10.4	17.8	7.8
The diseases of the respiratory system and organs	males	1.5	3.6	9.2	16.7	4.8
	females	5.1	6.9	9.5	2.2	6.6
The diseases of the nervous system and sense organs	males	6.1	3.6	2.6	16.7	4.8
	females	8.7	4.0	7.7	4.4	6.4
Congenital anomalies	males	4.5	–	1.3	–	1.9
	females	0.7	0.6	–	–	0.4
Others	males	10.6	14.5	13.2	8.3	12.6
	females	10.8	13.9	13.8	17.8	13.3
Totally	males	32.4	24.1	39.6	21.1	30.7
	females	67.6	75.9	60.4	78.9	69.3

Note. The structure of the diseases is based on the diseases, according to which the students belong to a special medical group

MATERIALS AND METHODS

The research was conducted at Zhytomyr National Agroecological University. It involved the students of the Faculty of Veterinary Medicine, Ecology and Law, Engineering and Energetics, Forestry, Economics and Management, Accounting and Finance, Technological and Agronomy. The examination of applicants concerning the health status and physical fitness was conducted in 1992-2002. The evaluation of the disease incidence rate of first-year students was carried out from 2002 to 2019, according to the results of medical examinations using medical cards and doctors' conclusions. The medical examinations were conducted by doctors at the university's medical center and the municipal polyclinic. The main pedagogical experiment involved 681 students (209 males and 472 females, aged 17-22). The analysis, systematization, and generalization of the literary sources and medical records of students allowed us to form the general situation of the disease incidence of the students of the university and Ukrainian higher educational establishments. In the second stage of the study, a comparative analysis of the students' diseases was carried out and they were classified. At the final stage of the research (2019), the final analysis and interpretation of the obtained scientific data were made.

The research methods included theoretical methods (the analysis and synthesis of literary sources, the analysis of curricula, methodical support, and the quality of classes with the students of special medical groups, the study and analysis of the medical cards of students); empirical methods (pedagogical observations, the methods of mathematical statistics).

This study complies with the ethical standards of the Act of Ukraine "On Higher Education" No.1556-VII dated 01.07.2014 and the Letter from the Ministry of Education and Science of Ukraine "On the Academic Plagiarism Prevention" No. 1/11-8681 dated 15.08.2018. Also, this study followed the regulations of the World Medical Association Declaration of Helsinki – ethical principles for medical research involving human subjects. Informed consent was received from all individuals who took part in this research.

RESULTS

The level of the health status of students depends significantly on the state of physical culture in the family and at the school, on following a healthy lifestyle, using the means of preventing diseases by young students that is the initial level of the health state of an applicant to a higher educational establishments. There has been a significant decline in health and in the level of physical fitness of students. The examination of the physical development and physical fitness of the applicants and first-year students of Zhytomyr National Agroecological University

during many years showed that more than 57.6 % of them had unsatisfactory physical fitness, significant physical disabilities, diseases; from 10.9 to 20.5 % students had an insufficient level of physical fitness, significant health disorders and they belonged to a group with reduced activity; from 8.1 to 17.4 % students belonged to a special medical group; from 0.4 to 1.2 % students were exempt from sports and had chronic diseases and disabilities (Table I).

The analysis of diseases, data of the physical development and physical fitness of applicants showed that from 24.0 to 30.8 % of them had diseases, including chronic, congenital anomalies, disability, very bad physical development (postural disorders, overweight and underweight, disproportionate physical development, problems with the functional abilities of an organism, etc.). When performing physical education tests and physical development assessments, a large number of applicants did not have the technical skills and knowledge about the necessity of such evaluations and of the performance of tests. In addition, a scientific analysis of the medical examination of school-age children conducted in Zhytomyr Region during fifteen years showed that the level of sick children ranged from 40.9 to 69.2 % [4, 19]. Accordingly, the low indicators of the health status and physical fitness of students are not accidental because university applicants are school graduates.

The analysis of medical examinations of first-year students carried out in 2003-2019 indicates negative tendencies in the health status of young people entering the universities of Ukraine. The main group included 74.3-89.7 % students, the special medical group involved 9.2-25.5 % students; from 0.1 to 2.2 % students had significant abnormalities in health status, chronic diseases, disorders of the musculoskeletal, cardiovascular, respiratory, nervous, endocrine systems, etc.

In 2017-2019, the Ministry of Education and Science of Ukraine modernized the system of physical education of university students, trying to bring it closer to the European level. First, the Departments of Physical Education were closed and the discipline of Physical Education was turned into an optional and independent form of study. At the same time, the material and technical support of Ukrainian universities is ten times inferior to the European universities, the mentality of Ukrainian students is such that they are not used to independent exercise, and there are no specialists in Ukraine who could quickly introduce modern European fitness technologies into the physical education process. All this began to destroy the Ukrainian system of physical education in higher educational establishments. Our research showed that the number of students who belonged to the special medical group was increased to 30.4 % in 2017, to 30.1 % in 2018, and the elimination of medical centers and polyclinics at universities and the lack of proper medical care for students led to an increase in the number of students in a special medical group to 44.1 % in 2019. This situation significantly worsened the quality of students' education, their level of physical fitness and working capacity.

Since 2009, 30.7 % male and 69.3 % female students belonged to a special medical group. A similar situation has survived to this day. There was also a clear tendency that a special medical group included more females than males. Regarding the structure of student disease incidence, it has significant differences among the authors [1, 5, 20, 21]. One of the reasons is the wrong approach to studying the structure of diseases. The students of the special education group most often have the diseases of the blood, blood-forming organs, circulatory system, musculoskeletal system (apparatus system and conjunctive tissue), urogenital and endocrine systems, vision, gastrointestinal tract, respiratory organs, organs of senses, nervous system, etc. Among the diseases of the respiratory organs, the most common are chronic bronchitis, pneumonia, bronchial asthma. Among the total number of diseases, cardiovascular diseases account for 37.2 % for males and 33.1 % for females; the diseases of musculoskeletal system account for 14.5 % for males and 12.5 % for females; urogenital and endocrine systems – 12.1 % for males and 12.1 % for females (Table II). Such diseases require the use of special health-improving physical exercises of a dynamic and cyclic nature. Physical activity should cause a small and moderate physiological response. To unload the cardiovascular system, it is possible to make exercises in a sitting position, lying, reclining, exercises with the raised legs.

The structure of the students' disease incidence is not actually changed during the period of study at higher educational establishments that is also confirmed by the data of other scientists [2]. The overall dynamics of the disease incidence of both males and females have some peculiarities. The disease incidence of males was decreased from 32.4 to 24.1 % in the second year, and on the contrary, the females' was increased from 67.6 to 75.9 % (Table III). There were also differences defined in the dynamics of the diseases of the students from different regions and higher educational institutions between the students of the 3rd and the 4th years [10].

The wide range of diseases and their quantitative and qualitative features require the departments of physical education to develop new methods of physical education, based on the principle of adequate physical activity, which corresponds to the level of health, characteristics of the disease and the interests of the students of special medical groups in different educational establishments. Therefore, we cannot but agree with scientists [18, 22] that the program strategy of the physical education of students should be based on a new paradigm of health, enriched with basic knowledge in the field of biology and health physiology, sport physiology, on the establishment of cause and effect dependencies between ordinary physical activity, mental capacity, and health, taking into account the psychological characteristics of the student's personality. And a student's organism, including all its components, such as physical, intellectual, moral, emotional, social, undergoes development and formation.

Accordingly, the methodical support of the health and fitness activity of the students of a special medical group should differ from the methodical system of the classes of the students of the main educational department not only in the amount of physical activity but qualitatively another physical activity. One of the quite efficient means of improving the quality of the educational process in a special medical group is the establishment of a clear system of assessment of students, the determination of criteria for exams in physical education. At the same time, the system of assessment must be humane, discrimination against an individual, limitation of one's dignity because of the different

Table III. The dynamics of the disease incidence of students during studying at higher educational establishments (n=681, 209 males, 472 females; % of the total number of diseases)

Gender	The years of studying			
	1st	2nd	3rd	4th
Males	32.4	24.1	39.6	21.1
Females	67.6	75.9	60.4	78.9

Table IV. Control tests and standards of assessment of the physical fitness of the students of a special medical group (males)

Tests	The criteria for assessing the improvement of the physical fitness indicators (the requirements and points)				
	5	4	3	2	1
3000 m race, min, s	–2.30	–1.55	–1.20	–0.40	e. l.*
100 m, s	–1.4	–1.0	–0.6	–0.3	e. l.*
Standing long jumps, sm	+35	+25	+15	+10	e. l.*
Push-ups, reps	+10	+8	+6	+4	e. l.*
Pull-ups, reps	+10	+7	+4	+2	e. l.*
Sit-ups, reps	+16	+13	+9	+6	e. l.*
4 x 9 m shuttle running, s	–1.2	–0.9	–0.6	–0.3	e. l.*
Forward reach from a sitting position, sm	+12	+10	+7	+3	e. l.*

Note. e. l.* – an entry level (initial datum is individual for every student)

levels of physical abilities are unacceptable. A teacher should be focused not on the results in the tests of physical fitness but the students' attitude to this type of activity, the level of motivation, the need for physical exercises after passing an exam, during vacations, after graduation.

At the same time, the standards in the educational process are necessary as a social norm of the physical fitness of students and the basis of the formation of the whole system of physical education. Otherwise, the physical education process will not be focused on health but may be reduced just to entertainment or outside activities. In order to enhance the attitude of students with disabilities and health defects to the process of physical education, we also developed and implemented control tests and standards for assessing physical fitness in a special medical group (Tables IV, V).

The standards are not a dogma for students but only a guide and an incentive to improve their initial level of physical fitness. The suggested system of physical fitness assessment requires the development of the scientific and methodological support of a new generation for the departments of physical education, more qualitative preparation of teachers for the work with students of special medical groups, the determination of special forms and the content of independent work and efficient means of monitoring the health of such students.

The other form of recovery activation and preservation of the health of the students of special medical groups is the systematic educational and practical work of a student with monitoring of the problems caused by one's disease by a teacher. In this case, a student learns the history of one's disease, the ways to behave in everyday life concerning the disease, dietary regime, the methods of treatment and organism recovery (pedagogical, psychological, hygienic, medical and biological, folk, etc.), forms the regime of motor activity, mentioning specific exercises, which one performs systematically at home, morning exercises, the complexes of exercises for physical activity breaks while doing homework. This is one of the tasks for passing an exam or a positive certification.

DISCUSSION

Our study reveals the reasons for the disease incidence and low physical fitness of the students of special medical groups, the analysis, and the classification of diseases. It is revealed that the main cause of a large number of diseases is the low level of motor activity of students, the physical condition and disease incidence of university applicants.

The following requirements must be considered for the development of the physical qualities of the students of special medical groups:

- to develop physical qualities only after reaching a sufficient level of functioning of the basic systems of an organism (respiratory, cardiovascular, nervous, etc.);
- to follow the sequence of the development of physical qualities: endurance, strength, speed. Flexibility and coordination skills can be developed from the first classes, taking into account the contraindications of individual students;
- to use moderate power exercises and physical activity, not to force the development of endurance when a student has an unsatisfactory state of the musculoskeletal system, excess body weight, obesity or abnormalities of the central and peripheral nervous system;
- during the development of power, maximum physical loads are not allowed. It is better to increase the number of repetitions of moderate exercise, to focus on the development of endurance;
- during the development of speed, special attention should be paid to the abnormalities of the musculoskeletal, nervous

Table V. Control tests and standards of assessment of the physical fitness of the students of a special medical group (females)

Tests	The criteria for assessing the improvement of the physical fitness indicators (the requirements and points)				
	5	4	3	2	1
2000 m race, min, s	–2.00	–1.25	–1.00	–0.25	e. l.*
100 m, s	–2,3	–2,0	–1,7	–0,8	e. l.*
Standing long jumps, sm	+40	+30	+20	+10	e. l.*
Push-ups, reps	+8	+6	+4	+2	e. l.*
Hanging on the crossbar with bent arms, s	+11	+9	+6	+3	e. l.*
Sit-ups, reps	+15	+11	+7	+4	e. l.*
4 x 9 m shuttle running, s	–1,5	–1,1	–0,7	–0,2	e. l.*
Forward reach from a sitting position, sm	+14	+11	+8	+5	e. l.*

Note. e. l.* – an entry level (initial datum is individual for every student)

and cardiovascular systems; to have a proper warm-up before performing the speed exercises;
- to combine physical exercises with respiration ones; to apply exercises with an optional duration of the respiratory cycle, aimed at improving the drainage function of the respiratory tract;
- to perform relaxation exercises, aimed at relaxing the muscles, alternating and combining tension and relaxation for the purpose of health-improving and recovery of the organism's functions after diseases.

It should be noted that only the organization of physical education, which engages both teachers and students in the management of the educational process, promotes the increased physical activity of students, deprives them of homogeneous traditional regulation. This makes it possible to change the position of students in the educational process, that is, to ensure that they become not only the object of the teachers' influence but also the subjects of their own activity, the equal participants in the management of the educational process. The initiatives make students individually and collectively responsible for the results of classes, sports and health-promoting events. The freedom of choice, variety of classes, maximum autonomy in decision-making, the desire to make their activities useful for themselves and the society are the vital conditions for the formation of the physical activity of the students of special medical groups.

The results of our studies confirm and supplement the conclusions of many scientists [1, 8, 10, 13, 15, 23, 24, 25].

CONCLUSIONS

1. The analyses and assessment of the state of students' disease incidence during the study at a higher educational establishments require the development of a modern scientific and methodical support for the process of physical education for the students of special medical groups, determining the special form and content of independent work and efficient means of controlling their health. Understanding the priority of the problem of the implementation of health-saving technologies in the educational process is crucial for the formation of the educational environment which will improve the students' health.
2. In order to activate the educational process and health and fitness activities of the students of special medical groups, it is necessary to interconnect all the components that may affect their quality and efficiency, namely: 1) to study an outlook, motives, interests, the attitude of students to physical education and health-improving activities; 2) to define the students' desire to overcome their health disabilities; 3) to identify individual characteristics of students, their level of knowledge about the causes of diseases and the physical development delay; 4) to substantiate the means, forms, methods, and principles of physical self-improvement; 5) to provide the maximum level of material and technical support of educational and health and fitness events for the students of special medical groups concerning their diseases and health abnormalities.

The prospects for further research are aimed at exploring the peculiarities of applying the means of physical health and fitness activities by the students with a low health level.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

THE EFFECT OF THE PRESENCE OF EPILEPTIC ATTACKS ON THE CLINICAL DURATION OF SUPRATENTORIAL BRAIN MENINGIOMAS

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ABSTRACT

The aim: To study the effect of epileptic seizures in patients with supratentorial brain meningiomas on the clinical course of meningiomas in the early and late postoperative period.

Materials and methods: A retrospective analysis of the course of the disease was performed in 242 patients with total removed supratentorial meningioma of the brain (general group). Long-term outcome of the disease was estimated in 176 people (a catamnesis group).

Results: The occurrence of a new neurological deficit was observed in 18 (18.0±3.8 %) patients out of 100 among patients with epileptic seizures before surgery and in 19 (13.4±2.9 %) out of 142 among those who had no seizures. The mortality rate was 1 (1.0±1.0 %) in the group of patients with seizures and 3 (2.8±1.4 %) in the group of patients without seizures before surgery.

The prevalence of new neurological deficits in the catamnesis group is 14 (19.2±4.6 %) of 73 patients with epileptic seizures before surgery and 17 (16.5±3.7 %) of 103 patients without seizures. Mortality was 3 cases (4.1±2.3 %) in patients with seizures and 9 cases (8.7±2.8 %) among patients without seizures.

Conclusions: No data have been obtained that the presence of epileptic seizures affects the incidence of new neurological deficits, complications and mortality after surgical treatment of meningiomas in the early and late postoperative period.

KEY WORDS: Epilepsy, meningioma, seizure, long-term follow-up

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INTRODUCTION

Meningioma is the most common intracranial tumour, with an incidence of 10.26 and 4.55 cases per 100,000 adults in women and men, respectively [1]. Often meningioma is manifested by seizures that significantly reduce the quality of life of the patient [2]. Surgical treatment is the method of choice in patients with newly diagnosed meningiomas [3]. Surgical treatment usually relieves the patient of the disease, but in some patients persists or occurs after the intervention of a particular neurological deficiency, as well as the possible complications of surgical treatment [4]. For patients with meningiomas who have a good long-term prognosis for life after surgery, this is especially important [5]. Research into the factors that influence the clinical course of meningioma after surgical treatment will optimize treatment tactics for this group of patients.

In 2017, a meta-analysis on the course of epilepsy in brain meningiomas reported that in the last 35 years, only 4 studies were directed to a detailed study of seizures in patients with meningiomas in pre- and postoperative periods [6]. Usually, studies have been performed that are more focused on determining surgical tactics and studying the predictors of prolonged growth than on determining the course of epileptic seizures. However, a number of publications have noted the prevalence and progression of patients. There have been a number of predictors of the persistence of seizures after surgery, such as swelling around the tumour and the male [7, 8, 9].

The study of the impact of epileptic seizures on the disease clinic in patients operated on for supratentorial meningioma will more accurately predict the course of the disease and, accordingly, may affect medical and surgical treatment. Previously, the authors analysed the group of patients with 110 people, but the study was more focused on the course of the epileptic seizures themselves [10].

THE AIM

The aim of the study was to investigate the effect of epileptic seizures in patients with supratentorialin brain meningiomas on the clinical course of the disease in the early and late postoperative period, with a view to further predicting the disease.

MATERIALS AND METHODS

A retrospective analysis of case histories of patients operated on for supratentorial brain meningioma was conducted at the Municipal Non-Profit Enterprise "Regional Clinical Center of Neurosurgery and Neurology" of Transcarpathian Regional Council, Ukraine from January 2006 to December 2017.

The study was approved by the local ethics committee of Municipal Non-Profit Enterprise "Regional Clinical Center for Neurosurgery and Neurology" of Transcarpathian Regional Counsil, Ukraine (protocol No. 115 of 12/18/2018) and performed in accordance with the provisions of the Declaration of Helsinki. All patients provided informed consent to the processing of their personal data in the study.

Table I. Basic clinical and instrumental characteristics of patients in the general group and the catamnesis group

Main group		Main group	Catamnesis group
Number of patients		242	176
Average age (years)		53.8 ±0.8	53.5 ±1.0
The distribution by sex	Men	79 (31.8±3.0 %)	56 (31.8±3.5 %)
	Women	163 (68.2±3.0 %)	120 (68.2±3.5 %)
The distribution by age	20–30 years	12 (5.0±1.5 %)	11 (6.3±1.8 %)
	31–40 years	22 (9.1±1.8 %)	19 (10.8±2.3 %)
	41–50 years	55 (22.5±2.7 %)	41 (23.3±3.2 %)
	51–60 years	75 (31.1±3.0 %)	44 (25.0±3.3 %)
	61–70 years	57 (23.6±2.7 %)	43 (24.4±3.3 %)
	71 and more	21 (8.7±1.8 %)	18 (10.2±2.3 %)
The distribution by localization	Upper-lateral surface	95 (39.3±3.1 %)	66 (37.5±3.7 %)
	Wings of wedge-shaped bone	60 (24.8±2.8 %)	46 (26.1±3.3 %)
	Sickle of the brain	51 (21.1±2.6 %)	42 (23.9±3.2 %)
	Hump of the saddle	13 (5.7±1.5 %)	10 (5.7±1.8 %)
	Perforating bones	15 (5.4±1.6 %)	8 (4.5±1.6 %)
	Lateral ventricle	5 (2.1±0.9 %)	3 (1.7±1.0 %)
	Lateral furrow	3 (1.2±0.7 %)	1 (0.6±0.6 %)
The distribution by histology	Grade I	197 (81.4±2.5 %)	138 (78.4±3.1 %)
	Grade II	34 (14.1±2.2 %)	28 (15.9±2.8 %)
	Grade III	11 (4.5±1.3 %)	10 (5.7±1.8 %)
The distribution by size (maximum diameter)*	Less than 30 mm	50 (24.8±3.6 %)	35 (23.3±3.5 %)
	30–59 mm	118 (58.4±8.3 %)	87 (58.0±4.0 %)
	More than 60 mm	34 (16.8±2.4 %)	28 (18.7±3.2 %)

Note: * – tumour sizes were analysed in 202 patients in the general group and 150 patients in the history group. The proportion was calculated in each individual distribution

Data on the clinical course of meningiomas before and immediately after surgery were obtained for 242 patients from whom a general group was formed. Long-term clinical outcome was evaluated in 176 patients who were referred to as a catamnesis group.

Inclusion criteria. Tumour localization over brain tent, primary surgery, and total tumour removal (Simpson I-III).

Exclusion criteria. Tumour localization below the brain tent, subtotal or partial removal, prolonged tumour growth, and multiple meningiomas.

All 242 patients underwent total removal of Simpson I-III meningioma. Each patient underwent postoperative neuroimaging control in the form of an MRI or CT scan.

Long-term treatment outcomes were evaluated in 176 out of 242 patients (72.7 % of the total group). It was decided to call this group of 176 patients a group of catamnesis. The average duration of observation was 37.0 months. The duration of observation was at least 6 months for each patient. The maximum duration of observation was 111 months.

The main clinical and instrumental characteristics of patients in the general group and the group of history are given in Table I.

All patients in the general group were divided into two subgroups. The first subgroup included 100 patients with brain meningiomas and concomitant epileptic seizures, and the second group included 142 patients with meningiomas who had no epileptic seizures before surgery. Diagrammatic distribution of patients is shown in Figure 1.

A subgroup of patients with epileptic seizures and a group of patients without epileptic seizures were compared. Whether the presence of epileptic seizures on the likelihood of developing neurological deficits and complications after surgical treatment in the early period has been investigated.

All patients of the history group were divided into two subgroups. The first subgroup included 73 patients with brain meningiomas and concomitant epileptic seizures before surgery, and the second subgroup had 103 patients with meningiomas who did not have epileptic seizures before surgery. Diagrammatic distribution of patients is shown in Figure 2.

A subgroup of patients with epileptic seizures (further group 1) and a group of patients without epileptic seizures (further group 2) were compared. It has been investigated whether epileptic seizures affect the likelihood of developing neurological deficits and complications in the long term after surgical treatment.

Statistical data processing was performed using Excel spreadsheets Windows-2007 license number 00426-OEM-8992662-00400, which is included in the Microsoft Office 2003 suite of programs, and STATISTICA license number ZZS999000009906307-DEMO5. Statistical analysis of

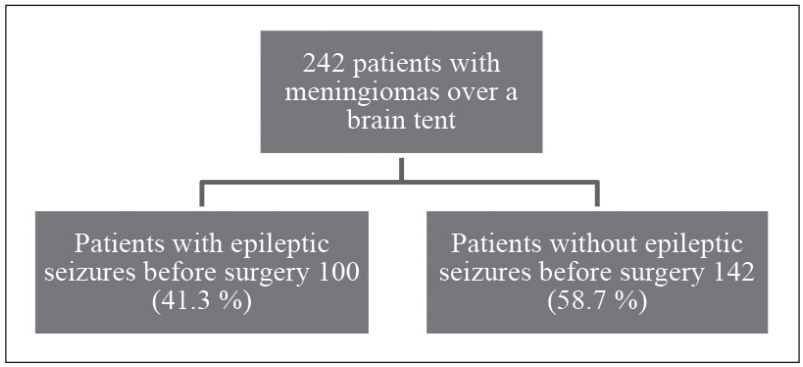

Fig. 1. The distribution of patients in the general group into subgroups

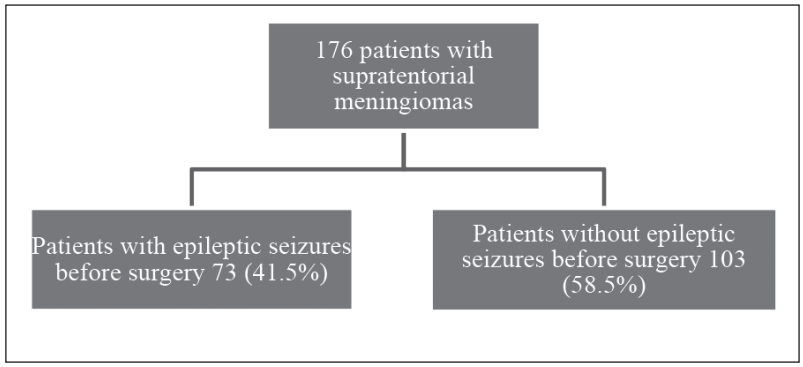

Fig. 2. Distribution of patients of the catamnesis group into subgroups

materials, summary of results and generalization of conclusions, performed by the method of variational statistics, taking into account the mean values (fashion, median, arithmetic mean) and mean error (M), also searched for a reliable difference between the groups (p).

RESULTS

At hospitalization, neurological deficits were presented in 113 (46.7±3.2 %) patients. At the time of discharge, neurological deficits persisted in 93 (38.4±3.1 %) patients, including 37 patients (15.2±2.3 %) that had a new neurological deficit. New neurological deficits were most often manifested by hemiparesis and speech disorders, but in the most cases, these symptoms were due to postoperative cerebral edema and gradually regressed. Improvement in neurological status was observed in 61 patients (25.2±2.8 %). In addition, it should be noted that one patient might have more than one clinical symptom.

Among patients who did not have a pre-intervention deficit, it occurred in 22 (17.1±3.3 %) patients out of 129. In 61 (51.3±4.5 %) of the 119 patients who had a neurological deficit before the intervention there was an improvement or the deficit regressed completely. A deficit increase was observed in 15 (13.3±3.2 %) cases out of 113.

Complications in the early postoperative period were in 9 patients, in 2 patients there were hematomas of the tumour bed, resulting in repeated surgery. One patient underwent reoperation for nasal liquor. The flap infection was in one patient and required surgical removal, followed by a skull bone defect. Another patient had pulmonary embolism on the 2nd postoperative day. Another patient with meningioma of the middle third of the sickle of the brain had a venous stroke in the early postoperative period, which resulted in a fatal outcome. Another patient with major menigioma also had a venous stroke followed by a fatal end in the region of the upper-lateral surface of the large hemisphere. In two patients with large meningiomas of the wing of the wedge bone, malignant ischemic stroke in the middle cerebral artery pool developed in the early postoperative period, leading to death. The total postoperative mortality was 4 (1.7±0.9 %) patients.

The comparison of early surgical results in the group of patients with epileptic seizures before surgery (100 patients) with the group of patients without epileptic seizures (142 patients).

Among patients with epileptic seizures prior to surgery, neurological deficits were observed in 29 patients out of 100, whereas in the non-seizure group, 84 out of 142. The proportion of patients with neurological deficits was 29.0±4.5 % and 59.2±4.1 % respectively, that is, in patients without epileptic seizures, neurological deficits occurred more often $p<0.01$. At the time of discharge, neurological deficits were observed in 29 patients in the epileptic seizure group and in 64 patients in the seizure group, which were 29.0±4.5 % and 45.1±4.2 %, respectively.

An increase in previous or new neurological deficits was observed in 18 patients from the group of patients with epileptic seizures, and 19 among them, who had no attacks, which was 18.0±3.8 % and 13.4±2.9 % respectively in each group.

Among 9 patients with early complications, 2 had patients who had epileptic seizures before surgery (1 case of stroke in the middle cerebral artery and 1 case of hematoma of the tumour bed), and 7 patients who had no seizures. Accordingly, the prevalence of early complications was 2.0±1.4 % among patients with seizures and 4.9±1.8 % in the group of patients without seizures.

Table II. Comparison of study groups regarding the early onset of additional neurological deficits, complications and mortality

	Group with epileptic seizures before surgery N=100		Group without epileptic seizures before surgery N=142	
	Number	Percentage	Number	Percentage
New deficit	18	18.0±3.8 %	19	13.4±2.9 %
Early complications	2	2.0±1.4 %	7	4.9±1.8 %
Postoperative mortality	1	1.0±1.0 %	3	2.8±1.4 %

Table III. Comparison of study groups on the retention of additional neurological deficits and long-term mortality

	Group with epileptic seizures before surgery N=73		Group without epileptic seizures before surgery N=103	
	Number	Percentage	Number	Percentage
New deficit	14	19,2±4,6 %	17	16,5±3,7 %
Mortality	3	4,1±2,3 %	9	8,7±2,8 %

It should be noted that among 4 deaths, 1 was in the group of patients with seizures and 3 in the group of patients without seizures before surgery. In percentage terms, it is 1.0±1.0 % and 2.8±1.4 %, respectively. Comparison of new neurological deficits, complications, and mortality rates between the study groups are shown in Table II.

It is clear that complications and postoperative mortality are higher in patients without seizures. This difference can be explained by the fact that neoplasms that are manifested by epileptic seizures are characterized by a smaller average tumour size, and rarely have another neurological deficit. In addition, overall, skull-based localization is less commonly associated with convulsions, and for meningiomas of the saddle, seizures in general are quite rare. However, no statistically significant difference between the groups revealed $p>0.05$.

When processing long-term results, it is noted that most neurological symptoms in patients gradually regress. In the distant period, 32 patients (47.8±6.1 %) of 67 patients who had a neurological deficit at the time of discharge were completely gone, but the situation is different for different neurological symptoms. Paresis was preserved only in 17 patients (37.8±7.2 %) of the 45 who had it at the time of discharge, i.e. it regressed in almost 2/3 of cases, whereas visual disturbances occurred only in 4 patients (26, 7±11.8 %) of the 15 who had them at the time of discharge. These results suggest that visual impairment is most predictably unfavourable to recovery.

The effect of epileptic seizures before surgery on neurological deficits in the early and long post-operative period was analysed.

Long-term paresis was observed in 5 of 14 patients who had them at the time of discharge in the group of patients with convulsions and in 12 of 31 patients who did not have a seizures before surgery. According to those patients who had paresis at discharge at the time of discharge, it was maintained in 35.7±13.3 % of persons in the seizure group and in 38.7±8.8 % among patients without seizures. With regard to other types of clinical symptoms, the dynamics were practically the same or the number of patients was small for qualitative statistical analysis and no significant difference was found between the study groups. The findings suggest that there is no difference in the recovery of neurological deficits, depending on whether or not there were epileptic seizures prior to surgery.

When determining the dynamics of neurological deficits, without differentiating between different clinical symptoms in the distant period, deficiency was present only in 32 (18.2±2.9 %) patients in 176. Most often, the lack of dynamics of neurological deficits was associated with visual impairment. The occurrence of a new neurological deficit or an increase in the previous deficit was usually seen in patients with vascular complications. Among 67 patients who had a neurological deficit at the time of hospital discharge, 32 (47.8±6.1 %) persisted for a long period.

Among 104 patients who did not have a neurological deficit before the intervention, in the long term, it was only found in 5 people (4.8±2.1 %). In contrast, 26 patients (36.1±5.1 %) of 72 had a deficiency among patients who had clinical symptoms before surgery. This could be explained by the fact that new neurological deficits are often due to postoperative edema or temporary vascular disorders, whereas neurological deficiency before surgery is often caused by an already formed organic CNS lesion.

Upon receipt of the catamnesis, 12 (6.8±1.9 %) of 176 patients were found to have died.

In 4 patients, death occurred as a result of various vascular complications in the early postoperative period, as described in detail in the section on evaluation of early results of surgical treatment. Another 4 patients died of cardiovascular disease (including one patient with myocardial infarction 2 months after meningioma removal). Two patients died of cancer. One patient died due to prolonged tumor growth. One patient died of a malignant tumor (not meningioma) of the brain.

It is resolved to compare long-term findings on neurological deficits and mortality among patients who had epileptic seizures before surgery (73 people in the catamnesis group) and did not have a trial before the intervention (103 people in the catamnesis group) (Table III). The table III, shows that in the long-term period new postoperative deficits were maintained at approximately the same level in both groups, while comparing the study groups for mortality, 9 patients were from the group

of patients without epileptic seizures, and 3 in the group of meningiomas with concomitant seizures. These data are most likely because patients with no convulsions were characterized by a higher incidence of early postoperative complications, but no significant difference was found between the groups.

DISCUSSION

The analysis of the clinical course of the disease in a large group of patients with supratentorial meningiomas in the early and late period after surgical treatment. Despite the significant difference in the groups regarding the prevalence of neurological deficit before surgery, there was no significant effect of the presence of epileptic seizures on the likelihood of neurological deficit, complications and mortality. On the other hand, performing the study on more patients can potentially lead to more recent data.

It should be noted that one feature is in the group of patients who had a neurological deficit before surgery. Who, at the time of discharge, had a neurological deficit assessed as "unchanged" in the future, usually had no improvement. Improvement was observed in those patients who at the time of discharge were already positive dynamics or, conversely, had a worsening in neurological status.

One of the major limitations of the study is the lack of analysis of the linkage of clinical complications with more than one factor, not just seizures alone. Perhaps some clinical sign in conjunction with convulsions will be prognostically unfavourable. This creates a potential field for new research.

A prospective study of the effect of epileptic seizures on the clinical course of supratentorial meningiomas in the brain is potentially needed.

It can be argued that the long-term results of surgical treatment are different from those at the time of discharge. In almost half of the patients who had a neurological deficit at the time of discharge, it disappears in the long term. The situation is better in the case of paresis, which disappears in approximately 2/3 of patients, and worse in the case of visual disturbances, in which the regression of symptoms occurs only in 1/3 of cases.

CONCLUSIONS

The long-term clinical results of surgical treatment of supratentorial meningiomas are significantly better than earlier. In almost half of the patients who had a neurological deficit at the time of discharge, it disappears in the long-term period. The situation is better in the case of paresis, which disappears in approximately 2/3 of patients, and worse in the case of visual disturbances, in which the regression of symptoms occurs only in 1/3 of cases.

No data have been obtained that the presence of epileptic seizures affects the likelihood of developing neurological deficits, complications and mortality after surgical treatment of meningiomas in the early and late postoperative period.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

STATUS OF ADOLESCENTS WITH SYMPTOMS OF IRON DEFICIENCY IN UKRAINE

DOI: 10.36740/WLek202003127

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ABSTRACT

The aim: The purpose of this research was to study the reasons adolescents are underdiagnosed in iron deficiency in Ukraine.

Materials and methods: We provided a data analysis of medical records on the reasons and frequency of requests for medical assistance among teenage children, including the age group10-19 years. The group of exclusion criteria included patients with traumatic brain injury, burns, eye diseases, congenital abnormalities of development, childhood infirmities, persons who had contact with radiation, oncological patients, patients who had surgery within the last 3 months. Moreover, the researched cases were provided by private medical institutions as we needed to compare the laboratory tests, which are not available in governmental primary care institutions. In this research were used such key methods as basic data analysis, correlation-analysis and summarizing of gathered results.

Results: The number of referrals dominated initially to ENT (36.8%), family physicians (25.6%) and pediatricians (23.3%), with a nearly equal distribution of patients by gender. From all the cases there were 162 cases where clinical laboratory blood test was performed (56,8% male, 43,2% female). The distribution among specialties of doctors, who prescribed a blood test was: 64,2% family doctors , 9,3%ENT, 20,4% pediatricians, 6,1%- other specialists. Serum ferritin was discovered in 22 cases. They were found in 19,1% and there was no correlation between inflammation changes and number of erythrocytes, hemoglobin and qualitative signs of red blood cells.

Conclusions: The analysis of medical records revealed the expediency of the syndromic distribution of causes of treatment, as well as the identification of risk groups and early diagnosis of pathologies among adolescent children in the practice of a family doctor. A fifth part of adolescent patients appointments was made up of healthy individuals, of whom (69.5%) belonged to the age group of 10-13 years. Inflammatory changes in the blood are not related to the amount of hemoglobin, erythrocytes and erythrocyte characteristics. Hemoglobin, hematocrit and erythrocyte counts are not informative in the diagnosis of iron deficiency.

KEY WORDS: iron deficiency, hemoglobin, hematocrit, adolescent.

Wiad Lek. 2020;73(3):546-550

INTRODUCTION

Family doctor (FD) faces a wide variety of pathologies in his everyday practice. Nowadays, in the realities of health care reform, primary care plays the leading and essential role in guiding patients to early diagnosting, treating, preventing diseases. Almost 80% of patients start and end their pre-hospital treatment not being carried to the secondary medical care. According to this fact, it is important to know risk groups of population to each abnormal condition of health. WHO provided a report "Health for the world's adolescents" fully addressing that question across the broad range of health needs of people ages 10–19 years and found them as a core area for health sector action. [1-3] National Center of Statictic in Ukraine shows, that in 2019 year there are nearly 13 million in rural areas and 29 million in cities of population, where part of people aged 10-19 years amounts 750 thousand in rural areas and 1,5 million in cities. For last 10 years morbidity in Ukraine remains nearly at the same levels. Adolescents usually get medical care in pediatricians or family doctors and the volume of diagnostical tools and services can differ depending from territory of living, region, whether it is governmental or private clinic, and for sure varies depending on knowledge and skills of doctor. Iron deficiency and anemia is one of the most common public health difficulties in the world and in Ukraine as well. [1-4] ID is associated with intencive cognitive function, impaired physical work capacity, physiological changes in hormones and poor pregnancy outcomes. The onset of menstruation in girls, rapid growth with phycological conditions that occur during adolescence, and the consumption of predominantly plant-based diets with low bioavailable iron, as well as product which tend to decrease iron consumption, all contributes to the depletion of iron stores that substantially increases the risk of teenage population to ID. What is more, the compliance to oral iron supplementation keeps quite low posissions because of side-effects of medicines, therefore it is important to diagnose ID as early as possible. [3, 5]

THE AIM

The purpose of this research was to study the reasons adolescents are underdiagnosed in iron deficiency in Ukraine.

MATERIALS AND METHODS

We provided a data analysis of medical records on the reasons and frequency of requests for medical assistance

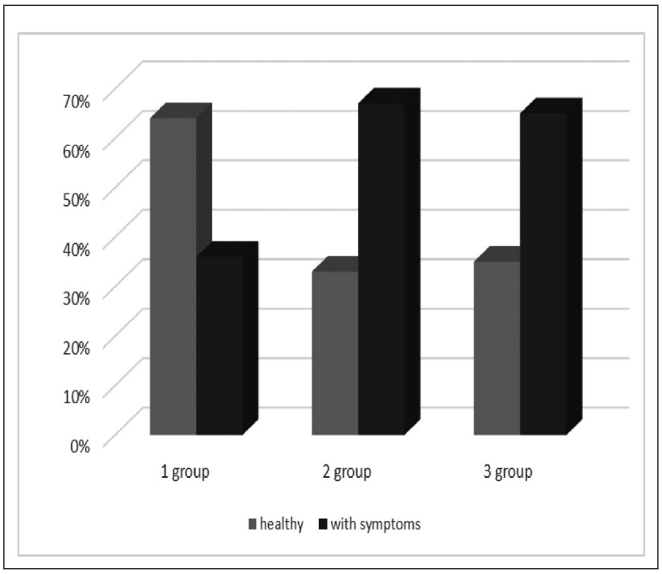

Fig. 1. Distribution among generally healthy teenagers and with deseases.

among teenage children, including the age group 10-19 years in Kyiv during 2018. The group of exclusion criteria included patients with traumatic brain injury, burns, eye diseases, congenital abnormalities of development, childhood infirmities, persons who had contact with radiation, oncological patients, patients who had surgery within the last 3 months. Moreover, the researched cases were provided by private medical institutions as we needed to compare the laboratory tests, which are not available in governmental primary care institutions.

In this research were used such key methods as basic data analysis, correlation-analysis and summarizing of gathered results.

The work was performed in accordance with the principles of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with the participation of a person as a research object" and approved by the Bioethics Commission of Shupik National Medical Academy of Postgraduate Education.

RESULTS

According to the World Health Organization (2007) anemia in adolesents is defined as hemoglobin level <12.0 g/dl. in female after 11 y.o and <13.0 g/dl in men in the age-group after 15 y.o. Serum ferritin level <15 μg/L and soluble transferrin receptor (sTfR) level <5.0 mg/L were considered as iron deficiency status.[6-7]

There were 725 cases of the primary application to the doctor for medical aid 390 (53.8%) female and 335 (46.2%)

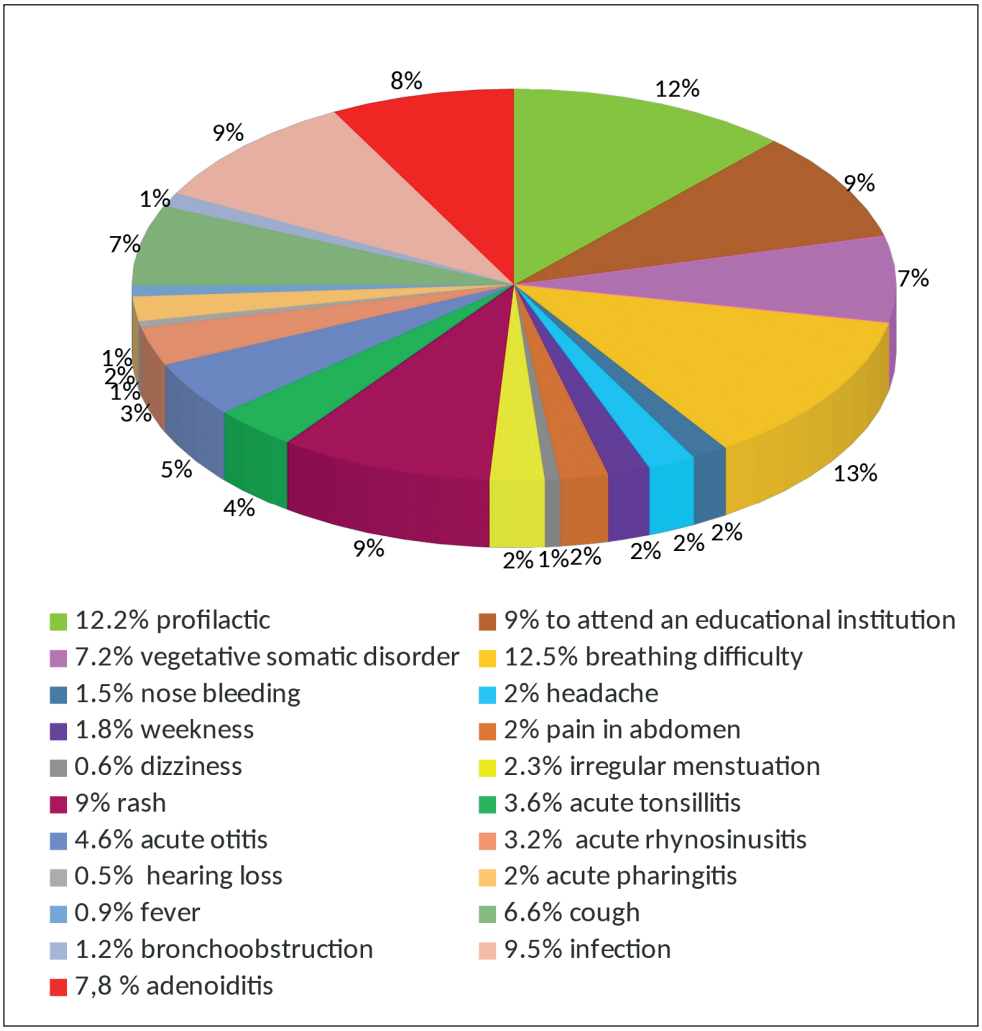

Fig. 2. Causes for appointment to doctor in patients aged 10-19 y.o.

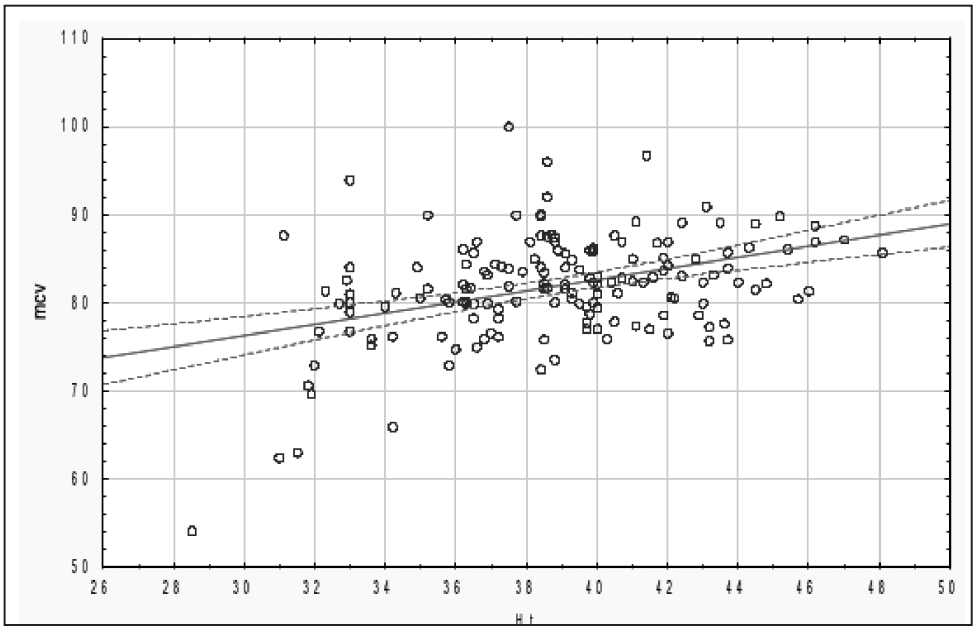

Fig. 3. Correlation between Ht and MCV

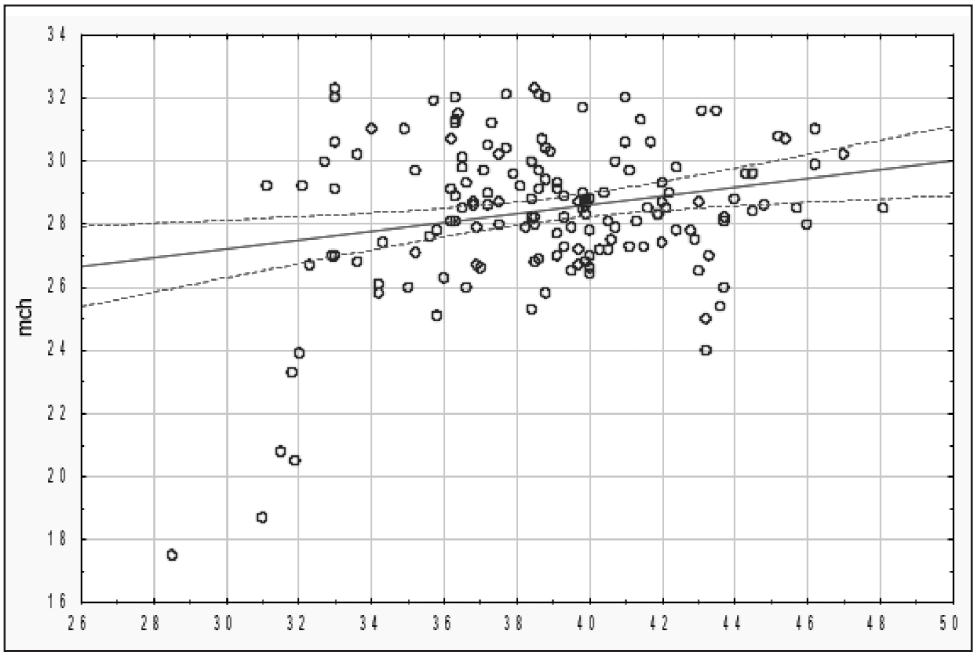

Fig. 4. Correlation between Ht and MCH

male 2018. The distribution according to the age was: early adolescent age (10-13 y.o.) group 1 – 338 cases (185 female, 153 male), middle adolescent age (14-17 y.o.) group 2 – 365 cases (189 female, 176 male) and late adolescent age (18-19 y.o.) group 3 – 22 cases (16 female, 6 male). The comparison above shows approximately equal percentages of male and female adolescents in groups 1 and 2. More than fifteen times less than among adolescents of late age. All the reasons of primary appointment to doctors were divided into two categories: mostly healthy individuals who need a medical conclusion for some activities or attending educational institutions or patients who needed a compulsory annual medical examinations because of complaints. We found that the quantity of in generall healthy teenagers was among all 21.2%, who mostly were people from group 1 (early adolescent age). The primary morbidity and acute pathology were higher in the 2nd group, but in general in numbered 57,6%. It is notable, that morbidity in the 3rd group was nearly the same as in 2nd one. (Figure 1)

The number of referrals dominated initially to ENT (36.8%), family physicians (25.6%) and pediatricians (23.3%), with a nearly equal distribution of patients by gender. There was a difference of 29.4% with a predominance of male among the consultations with a neurologist, 9% with a predominance of a female with a referral to a gastroenterologist and similarly with 12.4% with a referral to a dermatologist.

From all the cases there were 162 cases (22,3 %) where clinical laboratory blood test was performed (56,8% male,

43,2% female). The reason why they've got referrals were 78 patients (48 %) – routine yearly examination, 11 patients (6,79%) – clinical iron deficiency symptoms, 73 (45,21%) on other reasons or suspicion to inflammation. (Figure 2)

The distribution among specialties of doctors, who prescribed a blood test was: 104 (64,2%) family doctors, 15 (9,3%) ENT, 33 (20,4%) pediatricians, 10 (6,1%)- other specialists. Serum ferritin was discovered in 22 cases (13,5%). Inflammatory changes were considered if the number of leukocytes was high and erythrocyte sedimentation rate higher than 15 mmol/l. They were found in 31 cases (19,1%) and there was no correlation between inflammation changes and number of erythrocytes (Er), hemoglobin (Hb) and qualitative signs of red blood cells. MCV< 80 was found in 45 cases, of these, the hemoglobin was lower then 120 g/l in 7 cases, Er under normal age point – in 2 cases. MCH< 27 was found in 35 cases, of these, the hemoglobin was lower then 120 g/l in 11 people, Er under normal age point – in 1 case. Analyzes containing hematocrit and color index were obtained in 79 cases (48.76% of all analyzes), in other cases there was no color index and hematocrit. The result distribution: : hemoglobin 135 g / l (± 15.5), erythrocyte count 4.9 (± 0.67), hematocrit 39.57 (± 4), platelet count 264 (± 68.5), leukocyte count 7.39 (± 2.79), MCV 81.6 (± 6.59), MCH 27.95 (± 2.47), color index (CP) 0.84 (± 0.07). (Figure 3, figure 4)

According to the correlation analysis of indicators of clinical blood analysis revealed that there is a direct strong correlation between hemoglobin level and hematocrit (r = 0,94), a direct significant correlation between hemoglobin level and MCH (r = 0,63) and color index (r = 0.63), a direct moderate relationship between hemoglobin level and erythrocyte count (r = 0.45) and MCV (r = 0.49). There is a direct very strong correlation between color index and MCH (r = 0.93) and MCV (r = 0.9), a direct strong relationship between MCH and MCV (r = 0.88), a direct significant relationship relationship between hematocrit level and MCH (r = 0.58), MCV (r = 0.56) and color index (r = 0.57).

DISCUSSION

This research was conducted as a part of PhD research in social medicine according to the topic of iron deficiency screening . Sample size was considered as significant if there were more then 400 cases discovered. The exclusion criteria patients with traumatic brain injury, burns, eye diseases, congenital abnormalities of development, childhood infirmities, persons who had contact with radiation, oncological patients, patients who had surgery within the last 3 months. Randomization was generated hence we provided our analysis in different districts, didn't deal with patients, we only worked with their former conclusions, during 2018.

The results of correlation analysis should be proved in a bigger sample size and to compare the results with ferritin level, as well as it's essential to prove the results in different regions of Ukraine.

The analysis of medical records revealed the expediency of the syndromic distribution of causes of treatment, as well as the identification of risk groups and early diagnosis of pathologies among adolescent children in the practice of a family doctor. There is a tendency for the highest number of complaints about diseases of the upper respiratory tract (46.4%), skin and subcutaneous tissue (9%), vegetative somatic disorders (7.2%). A fifth part of adolescent patients appointments was made up of healthy individuals, of whom (69.5%) belonged to the age group of 10-13 years. In the age group of 14-17 years the figure was (28.7%). The majority of appeals among children aged 10-19 were appeals to the ENT doctor (36.8%), family doctor (25.9%), pediatrician (23.3%). The distribution among specialties of doctors, who prescribed a blood test was: 104 (64,2%) family doctors, 15 (9,3%) ENT, 33 (20,4%) pediatricians, 10 (6,1%)- other specialists. Serum ferritin was discovered in 22 cases (13,5%).

CONCLUSION

Inflammatory changes in the blood are not related to the amount of hemoglobin, erythrocytes and erythrocyte characteristics. Hemoglobin, hematocrit and erythrocyte counts are not informative in the diagnosis of iron deficiency. Consequently, patients of the adolescent age need to be yearly referred to the clinical blood test with checking ferritin if there are any risks of iron deficiency. In the case, when there are no MCH and MCV mentioned, it is possible to consider color index as marker of possible iron insufficiency.

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THE PSYCHOLOGICAL RESEARCH OF THE DOMINANT CONFIGURATION MODELS OF MARITAL RELATIONSHIPS OF A WOMEN WITH NEUROTIC DISORDERS

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ABSTRACT

The aim: To study the psychological peculiarities of relationships in families of women with neurotic disorders.

Materials and methods: 30 women who received treatment in the neurotic unit of the A.F. Maltsev Poltava Regional Clinical Psychiatric Hospital and 30 mentally healthy women have been examined. In the research the authors used a complex of interrelated and complementary general scientific and specific methods based on the systematic approach.

Results: There was a tendency for following types of marital interactions: dependent – 58% of couples, intermediate – 35% of couples. The emotionally-metaphorical type of interpreting life events is inherent for 89% of women with neurotic disorders. Studying the types of relationships with a husbands demonstrated that women with neurotic disorders perform 'the role of victim' – 30% (1,7689) and 'the role of 'burden" – 40% (1,7689) in their relationships.

Conclusions: The types of relationships with a partner of women with neurotic disorders are partly determined by the specifics of early parenting experiences.

KEY WORDS: neurotic disorders, relationship narratives, family narrative, dominant configuration in relationships, CCRT

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INTRODUCTION

Neurotic disorders rank among the most prevalent mental illnesses [1,2]. The study of the dominant configuration of relationships of women with neurotic disorders is very important in this sense. As the dominant configuration is the subjective relations of the individual, the formation of which is carried out under the influence of appropriate methods of perception of the environment, adequate or inadequate reactions to the environment, normal or pathological forms of a psychological defense. These are the keys to relationship with oneself, relationships with other people and relations with the environment.

These patterns originate in early childhood experiences, more precisely, in examples of family interactions that were often repeated. Such relationship models are effectively studied using the RAP interview method.

THE AIM

To find out and study the behaviour models in families of women with neurotic disorders.

MATERIALS AND METHODS

30 women who received treatment in the neurotic unit of the A.F. Maltsev Poltava Regional Clinical Psychiatric Hospital (the patients – the experimental group) and 30 mentally healthy women (the respondents – the control group; mean age of all women 40) have been examined. The women participated in a longitudinal study over a 1year period.

During the research, we used a complex of interrelated and complementary general scientific and specific methods based on the systematic approach. Such as the family sociogram, questionnaires and the Relationship Anecdotes Paradigm (RAP) interview.

Sociometric techniques are methods for observing, measuring and modifying social interactions, including family interactions. Sociometric techniques are well suited to couple and family therapy because of their effectiveness in vividly portraying each person's place in the system, the member's patterns of interaction and their feelings about it [3]. We used the family sociogram for charting preferences and choices in family relationships. The respondents were presented a handout with a circle with a diameter of 110 mm drawn on it. Then, the respondents were asked to draw themselves and their family in a circle. We analyzed the next criteria: the number of family members in the circle area; the magnitude of the circles; the arrangement of circles relative to each other; the distance between them.

The questionnaires method was used for researching the peculiarities of family relationships. It allowed to implement a subjective approach to psychodiagnosis and increase our understanding of respondents' family behaviors.

The RAP interview method is a purposeful conversation with the client about his or her relationship with others [4]. The respondents were asked to tell about specific cases of their interactions with others (for example, meeting, telephone conversation) that had taken place in the past. The cases would be specific, validated examples, and should not be limited to summarize the 'typical' situation of relationships with significant others. Since the materials of our research were transcripts of special interviews with patients and respondents, the relationship episodes were highlighted by us as particular stories that reflected relationships with significant others. The relationship episodes include three components, such as: (a) wishes, needs, or intentions expressed by the subject (wishes); (b) expected or actual responses from others (ROs); and (c) responses of self (RSs); i.e., the patient's own emotional, behavioral, or symptomatic responses to responses of others [5]. Then components received from all processed episodes were analyzed, reformulated in conformity with CCRT and statistically calculated.

RESULTS AND DISCUSSION

In the present study, the family sociogram found out the following types of interaction in the studied pairs of women with neurotic disorders: dependent – 58% couples, intermediate– 35% couples, harmonious –7% couples.

The most popular type of family interaction among the researchers is *dependent*. *Dependent pattern of interaction* is characterized by the following features: excess value relation to family members with fixing family relationships, unrealistic, uncritical expectation of unconditionally positive attitude on the part of family members to each other, with the refusal of an opportunity to be themselves; conscious fear of being abandoned, which reduces negative feelings about objects and forces any victims to preserve relationships; unconscious fear of intimacy between the couple that inhibits sex appetence in physical intimacy , the desire to be with another person and at the same time fear to be absorbed by him.

For families with such type of relationship imbalance in the hierarchy between parent and child subsystems are inherent. This option can be represented as a wheel where the spokes are children, and axis is the mother, who is responsible for every little thing that happens to children without the transference of some responsibilities to them. Such mother seems to be overloaded with her permanent tasks. Children always ask something their mother, show her what they have done, ask her to arrange conflicts and try to consult everything before doing something. Such structure is typical for unshared power.

Intermediate pattern of interaction that is inherent in 35% of the studied couples is characterized by the following features: configuration of relationships is intermediate, communication of family members is realized through a third person (for example, child) or through a common theme (for example, family business or family member illness), thus, communication does not occur directly, and not all members of the nuclear family are directly related. Married couples with this type of interaction cannot discuss openly their problems and the tension in their relationships. It means, the regulator of family relationships is beyond area of the family, it's in the expanded system of relations.

The harmonious pattern of interaction, which is least characteristic of the studied couples, is characterized by the following features: relationships in nuclear families are strong and medium, the emotional distance between the members of the nuclear family is close; boundary of the nuclear family is normal, it means that there is sufficient exchange with the environment, there is a good balance between 'take' and 'give'; with enough intense emotional ties, there is enough autonomy for each family member and children. For example, the spouses have common friends and common interests, but they have their own friends and interests as well. Due to this a common and a private area are saved. Responsibilities are shared, responsibilities are delegated. *These families also experience the drift between the parental 'control' and 'risk' for their children's autonomy.*

The next step in our study was to analyze the way the patients experience and interpret their own lives, through the RAP interview method.

O. Sapogova [6] suggested a formal analysis of narratives and distinguished the following methods of organizing narratives about events:

- *The ascertaining emotionless method (the descriptive method)* is a schematic list of events, statement of facts without their analysis, emotional impressions and reflections; the superficial narrative;
- *The emotionally metaphorical method (the performative method)* is a description of 'cases' (lyrical, comic, tragic, dramatic) that have a special meaning and significance; narratives are accompanied with evaluations, questions and etc.;
- *The instructive method (the didactic method)* the story is composed or saturated with instruction, edification, instructive statements as an 'example' of a courageous or other, worthy imitation of life; the description consists of many conclusions and moralizing statements;
- *The analytical and teleological method (the entimematic method)* is a description of purposeful movement towards the goal with analysis and explanation of the motivation of own actions or actions of other people, as a confrontation with obstacles;
- *The symbolic method (the allusive method)* is a description of each life event as mystical that has the secret meaning, influence on the destiny, on other people, on the world in general.

This study found that significant differences exist between the study groups. In particular, the most prevalent type of interpretation of life experience among control group is the ascertaining emotionless method – 65% ($p \leq 0,05$). The respondents are characterized by emotionless, impartial, and the most objective attitude to life events. They try to build their attitude to life as objectively as possible, in contrast to the experimental group under study, where the emotionally metaphorical method interpretation is the leading type of narratives – 89% ($p \leq 0,01$).

The emotionally-metaphorical type of interpreting life events is inherent for 89% of women with neurotic disorders. Thus, patients are inclined for symbolic interpretation of life events, using metaphorical statements in their description and moving away from the objective evaluation of life events.

Other ways of interpreting life events among the groups are not well expressed. The instructive method was found in 6% of women of the experimental group and in 15% of the control group. It means, the interpreting of life events by the instructive method is little inherent in patients.

The analytical and teleological method was revealed in 20% of women of the control group and 5% of the experimental group. These results suggest that both patients and respondents are not inclined to a detailed and balanced analysis of life events and their impact on the present, but respondents have a certain tendency for that.

So, this part of research demonstrated that patients are really more focused on the emotional interpretation of the events of their lives and their uncritical perception. But the respondents are more focused on the schematic list of events of their lives without emotional impressions and building narratives on that basis.

Studying the types of relationships with a husbands demonstrated that women with neurotic disorders perform *the role of victim* – 30% (1,7689) and *the role of 'burden'* – 40% (1,7689) (the dependent interaction) in their relationships. That is, most of women feel addicted to their husbands, relying on husbands' opinion in all matters and exhibiting psychological immaturity; or consider themselves victims of their husbands. To a lesser extent, patients are also characterized by such types of relationships as *'aggressive type'* – 13% and *'conflict with the desire to distance'* – 13,7%. The least popular types of relationships with a partner are 'harmonious' – 0% (completely alien to women with neurotic disorders), and 'conflict with a desire to become closer' (3,3%). So, women with neurotic disorders are inherent neither harmonious relationships nor the desire for psychological closeness with a husband.

The relationships with husbands have emotional primitive nature in the experimental group as opposed to the control group where the high rates of positive relationships (13.8489) indicate their high emotional color.

The qualitative analysis of the interview within the CCRT results shows that the reactions of other people usually are described by the experimental group more negatively than their own reactions – 80%.

In general, the results of the RAP interview indicate that the most often CCRT occurred in patients are *the desire for love, the social rejections* and *their own disappointment reaction.* A low level of the desire *'to have understanding with others'*, *an increased level of dislike for others* and *feelings of hopelessness and helplessness* were revealed in over half of the patients of the experimental group.

The patients tend to the typical patterns in relationships that are characterized by *a low desire to get help from others* and *a less desire to communicate with others to improve their own well-being.* Women with neurotic disorders are characterized by *alexithymia* and *anhedonia* in relationships. So, the behaviour patterns specific to alexithymia had been identified: *the need to be kind to others* is reduced; *perceiving others as willing to help*; l*ow response to the needs of others* and *conflict with somatic symptoms.* Therefore, women with neurotic disorders have *low expectations about other people* and *have no motivation to excuse the expectations of others.* These results are fundamentally different from results of the control group.

Based on the narrative analysis, we have hypothesized that the characteristics of relationships in the parental families of the women with neurotic disorders are the basis for creating models of relationships with their own husbands. The peculiarities of the influence of relationships with parents on the marital interaction of the investigated have been determined also using the CCRT method. From the point of view of psychoanalysis, such relationships are the result of the relationship of personal and individual desires, fears and protective strategies, on the one hand, and the reactions and models of partners, on the other [7].

This study had found that patients demonstrate all types of relationships (conflict with the desire to distance, conflict with the desire to become closer, the role of victim, aggressive type, dependent type) with their parents, except the harmonious ones. Each type of relationships is inherent to 20% of experimental group. 20% patients are characterized by such type of relationships as '*the conflict with the desire to become closer'* and 20% patients as – *'the conflict with the desire to distance'*. The parent-child relationships of women with neurotic disorders are conflictual, but in the first subgroup due to excessive distance (in this case, women want to become closer to their parents) and in the second subgroup due to excessive parental involvement in children's lives (in this case, women want to set the family boundaries and to be separated from their parents). The dependent type of relationships with their parents is inherent for 20% of the patients. That is, they felt a total dependence on parents which caused them to manifest of psychological immaturity, dependence etc. 20% of those who were interviewed indicated that they performed 'the role of victim" in the parent-child relationships'. They felt victimized and developed a proper behavior (behaving as if all family troubles had arisen because of their presence in the family, periodically manipulating parents). Also 1/5 part of experimental group is characterized by the aggressive type of relationships with their parents: patients felt constant reproaches from their parents, psychological distance from the parents and had been physical abused in childhood.

Thus, in part of patients their autonomy was maintained in the parental family because of their parents' indifference to them. Relationships were maintained through the control or over-involvement of parents in the child's life. This formed an immature relationship model dominated by interconnections, interdependence, and fear of intimacy.

The passive desires of patients *('I want to be loved and understood')* dominate in episodes of relationships with their parents (mother, father) – 81%; whereas on the contrary the active desires *('I want to love, I want to become*

to closer him') dominate in episodes of relationships with husbands – 70%. The father is most often described as dominant – 84%, while husband – as controlling – 68%. Other women are more often characterized as strong and not ready for understanding – 75%. But, in general, all patients feel respect and love of their parents more often than the same of their husbands – 91%. Also patients have less control over themselves in the relationships with husbands.

The types of relationships with a partner of women with neurotic disorders are partly determined by the specifics of early childhood experiences. At the same time, the share of positive reactions in relations with husbands exceeds the share of positive reactions towards parents in both groups: the experimental group – 85%, the control group – 60%.

The results of our study showed that patients have the ability to change cliché-shaped relationships models; and have the ability to form new behavioral patterns in a current relationship. Patients can use the positive experience of the relationship as a positive identification and a positive social reinforcement. Also the positive experience of the relationships can be used by psychiatrists, psychologists, psychotherapists to help determine patients' psychological resources; to evaluate patients' ability 'to think out of the box', to perceive and describe the current relationships differentially.

CONCLUSIONS

As a result, we have demonstrated that the dominant configuration actually has powerful influence to interaction not only with parents but interaction with other significant men and women for patients as well. Along with this, in the episodes of relationships with husbands' *other categories and models of relationships* that differ from parent-child come to the fore both of the patients and the respondents. Despite overwhelmingly negative relationships with parents and women's inclination to transfer these relationships to other people, women could change cliché-shaped relationship models and have a positive relationship with other significant partners. This indicates *important interpersonal resources* of psychotherapeutic patients and clients, meaning that they are more flexible than they seem, and capable of creating new types of relationships and experiences that they ever had with their parents. Besides psychotherapeutic patients and clients can enjoy the support of other people, if not completely, then not infrequently from close people and important social environment.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

IMPACT OF DIFFERENT ANGIOTENSIN-CONVERTING INHIBITORS ON OUTCOMES OF POST-MYOCARDIAL INFARCTION PATIENTS

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ABSTRACT

The aim: To compare the long-term effects of different angiotensin-converting enzyme inhibitors in post-myocardial infarction (MI) patients.

Materials and methods: Of 445 consecutive patients with myocardial infarction, 76 (17%) patients had co-morbid conditions, as well as were found to be compliant with secondary prevention treatment and eligible for follow-up. These patients were assigned to ramipril, perindopril or zofenopril groups in complex management of post-MI period. Subsequently, the patients were followed-up prospectively for a period of up to 24 months.

Results: Patients of zofenopril group performed better in terms of post-MI biventricular remodeling and left ventricular function recovery. Also, patients receiving zofenopril showed benefits in terms of short-term and long-term mortality as compared with patients of ramipril and perindopril groups.

Conclusion: Zofenopril may have advantages over perindopril and ramipril in the complex management of post-MI patients in terms of prevention of negative myocardial remodeling, onset of congestive heart failure and major adverse events.

KEY WORDS: myocardial infarction; angiotensin-converting enzyme inhibitors; myocardial remodeling; co-morbidities; secondary prevention

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INTRODUCTION

Myocardial infarcion (MI) is one of the most dramatic manifestations of coronary artery disease characterized by high mortality rates due to fatal arrhythmias, cardiogenic shock, pulmonary edema, as well as due to congestive heart failure (CHF). A significant number of multicenter randomized clinical trials (CONSENSUS II, SAVE, AIRE, ISIS-4, GISSI-3, CCS-1, SMILE 1-4, PREAMI, TRACE) have demonstrated that angiotensin-converting inhibitors (ACEI) have proven angioprotective properties and exhibit positive effects in terms of prevention of post-infarction myocardial remodeling and development of CHF. All ACEI are characterized by similar clinical effects due to neurohumoral blockade. Overall, the effect of any ACEI is predictable and considered as a "class effect". Nevertheless, each ACEI has its specific pharmacokinetic properties which potentially may influence the clinical outcomes. In most clinical trials different ACEI have not been directly compared. While choosing an ACEI in specific clinical scenario most physicians are guided by the available scientific evidence as well as by their personal experience. Currently most of the evidence-based data on the use of ACEI in MI are derived from the studies of ramipril [1], perindopril [2] and, in the past 10-15 years, zofenopril [3-6].

THE AIM

To compare the long-term effects of different ACEI in post-myocardial infarction patients with co-morbidities who were adherent to secondary long-term prevention.

MATERIALS AND METHODS

A total of 455 patients (342 [75,16%] – males) with ST-elevation acute MI aged 62,7±1,07 years gave informed consent for participation in the study. Depending on the prescribed ACEI, the patients were divided into three groups: group 1 patients (n=232, 51%) were receiving ramipril 2.5-5,0 mg qd, group 2 patiens (n=171, 38%) were prescribed perindopril 2.0-4.0 mg qd, group 3 patients (n=52, 11%) were administered zofenopril at initial dose of 7.5 mg bid with subsequent dose uptitration to 60 mg qd. Differences in the number of patients between the groups is due to variations of government supply of different ACEI foe acute MI patients. The choice of an ACEI was largely influenced by ACEI availability dependent on government supply. Comorbidities were assessed with the use of Charlson Comorbidity Index and CIRS index. Adherence with treatment recommendations was of patients was assessed according to Morisky-Green questionnaire as previously described [7]. Only compliant patients were selected for the study. These patients were followed-up prospectively for a period of up to 24 months. Serial echocardiographies, laboratory evaluations (NT-proBNP) as well as adverse events analysis (recurrent myocardial infarction, cardiovascular death) were preformed during follow-up. The study was approved by the local ethics committee.

RESULTS

In our previous study [13] we have analyzed adherence to long-term treatment with ACEI. Unfortunately, only 76 (17) % of patients with comorbidities were found to be sufficiently adherent with treatment (Morisky index ≥3), and, therefore,

Table I. Baseline characteristics of patients compliant with treatment recommendations

Variables	Group I, n=36	Group 2, n=21	Group 3, n=19
	n, (%)	n, (%)	n, (%)
Gender	26 (72.22)	13 (61.90)	15 (78.94)
MI subtype			
Anterior MI	13 (36.12)	10 (47.62)	12 (63.15)
Inferior MI	12 (33.33)	6 (28.57)	5 (26.32)
Recurrent MI	7 (19.44)	3 (14.29)	2 (10.53)
Non-Q-wave MI	4 (11.11)	2 (9.52)	
Charlson index CIRS index	4.95±0.15 9.40 ±0.16	5.50±0.24 10.01±0.27	5.75±1.10 10.25±1.43
Comorbidities			
No comorbidities	2 (9.52)	6 (16.67)	3 (15.79)
Gastropathies	3 (14.29)	4 (11.11)	6 (31.57)
Smoking	5 (23.81)	10 (27.78)	4 (21.05)
COPD	5 (23.81)	7 (19.44)	4 (21.05)
Arterial hypertension	16 (76.19)	22 (61.11)	13 (68.42)
Diabetes mellitus	4 (19.04)	8 (22.22)	3 (15.79)
Arrhtythmias			
Supaventricular extrasystoles	12 (57.14)	17 (47.22)	8 (42.10)
Ventricular extrasystoles e	6 (28.57)	10 (27.78)	4 (21.05)
Supraventricular tachycardia	2 (9.52)	3 (8.33)	3 (16.7)
Ventricular tachycardia	-	2 (5.55)	-
Atrial fibrillation	5 (23.81)	5 (13.88)	2 (10.53)
Ventricular fibrillation	-	2 (6.7)	-
LBBB	1 (6.3)	2 (18.2)	-
RBBB	2 (13.3)	2 (18.2)	-
2nd degree AV block	-	-	1 (9.1)
Complete AV block	-	-	-
Cardiogenic shock	2 (11.8)	1 (9.1)	-
Pulmonary oedema	-	1 (9.1)	-
LV aneurysm	-	1 (9.1)	-
Acute HF Killip I	-	6 (54.5)	-
Acute HF Killip II	-	3 (27.3)	-
PCI	21 (58.33)	35.3 (57.14)	15 (78.94)

No statistically significant differences between groups were found ($p > 0.05$).
AV = atrio-ventricular, COPD = chronic obstructive pulmonary disease, HF = heart failure, LBBB = left bundle branch block, LV = left ventricular, RBBB = right bundle branch block, PCI = percutaneous coronary intervention

they have been selected for the study. Baseline characteristics of patients who were found to be compliant with the prescribed therapies were not statistically different from those of non-compliant patients. Similar baseline characteristics were observed in all 3 study groups (Tables I, II): no significant differences were found between the morphometric and functional indexes of group 1-3 patients with optimal adherence.

Serial NT-pro BNP measurements were performed within the first 3 months of follow-up (Figure. 1). In the ramipril group (group 1) the acute period of MI was characterized by a rapid growth of NT-proBNP levels. Similar, although less marked tendency was observed in the perindopril group (group 2). Only in zofenopril group (group 3) a gradual steady decrease of NT-proBNP levels was observed since the first days after acute MI. After 3 months follow-up, NT-proBNP levels were decrease in all three goups, the decrease being statistically significant only in zofenopril group (group 3).

Different impact of ACEI on postinfarction myocardial remodelling was assessed by serial echocardiographic exams. The analysis was performed within the early and long – term

Table II. Baseline morphological and functional characteristics of patients

Variables	Group I, n=36	Group 2, n=21	Group 3, n=19
RV/LV ratio	0.72±0.02	0.77±0.03	0.66±0.02
RWT	0.43±0.02	0.51±0.02	0.42±0.01
LV EDDI, cm/m^2	2.74±0.06	2.71±0.06	2.72±0.07
RVI, cm/m^2	1.24±0.04	1.23±0.05	1.23±0.05
LVMI, g/m^2	137.95±7.44	142.12±6.36	138.05±5.57
FS, %	24.11±1.07	26.17±0.79	26.10±1.49
LVEF, %	47.09±1.59	48.65±1.31	46.18±1.22
NYHA class	2.00±0.14	2.11±0.20	1.67±0.33

No statistically significant differences between groups were found (p >0.05).
FS = fractional shortening, LV = left ventricular, LVEF = left ventricular ejection fraction, LVMI = left ventricular mass index, NYHA = New York Heart Association, RV = right ventricular, RVI = right ventricular index, RWT = relative wall thickness.

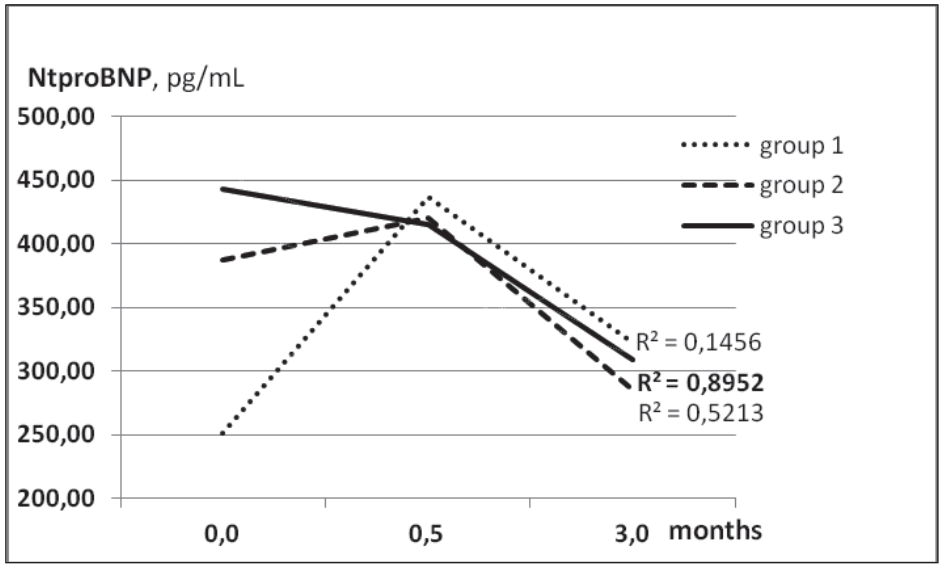

Fig. 1. Serial changes of N-terminal pro-brain natriuretic peptide (NT-proBNP) within the first 3 months after acute myocardial infarction.

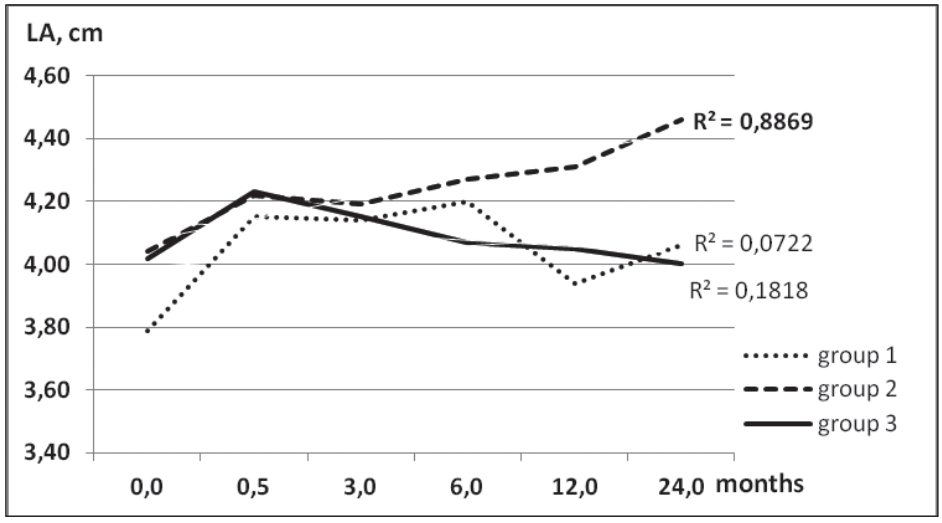

Fig. 2. Serial changes of left atrial, left and right ventricular dimensions during follow-up. LA = left atrial antero-posterior dimension, LV EDDI = left ventricular end-diastolic dimension index, RVI = right ventricular index.

(up to 24 months) period. As compared with perindopril (group 2), the use of zofenopril was effective in terms of prevention of postinfarction LV and/or left atrial enlargement. No increase of left atrial antero-posterior dimension (Figure 2) or left atrial volume was observed in the ramipril and zofenopril group. In patients receiving perindopril (group 3), significant left atrial dilatation was observed by the end of follow-up.

Analysis of serial changes of ventricular dimensions revealed gradual increase of end-diastolic dimensions of both ventricles, especially of the right one, within 24 months follow-up in patients who were administered ramipril and perindopril. In complex therapy with the use of zofenopril the LV end-diastolic dimension index decreased significantly; similar changes were observed in the right ventricle as well. (Figure. 3, 4).

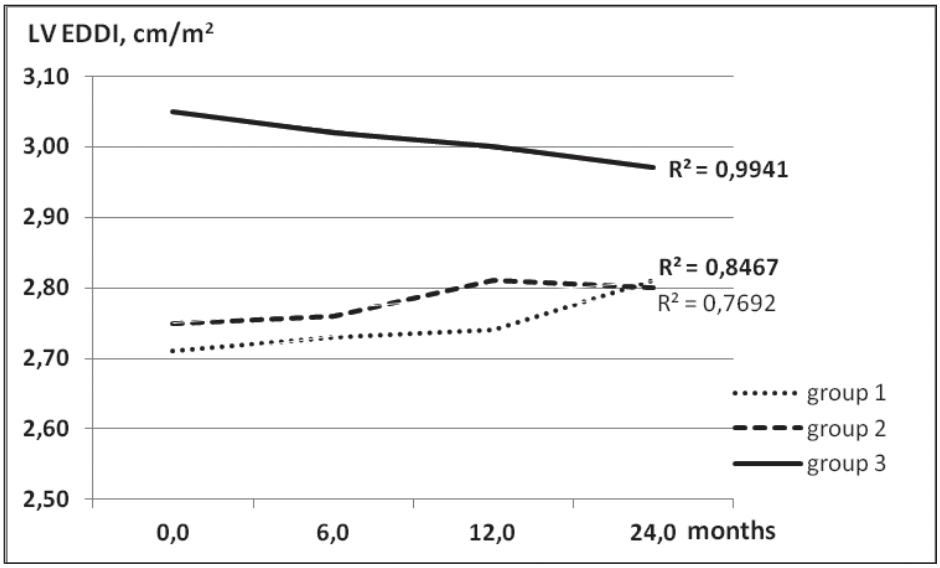

Fig. 3. Serial changes of left atrial, left and right ventricular dimensions during follow-up. LA = left atrial antero-posterior dimension, LV EDDI = left ventricular end-diastolic dimension index, RVI = right ventricular index.

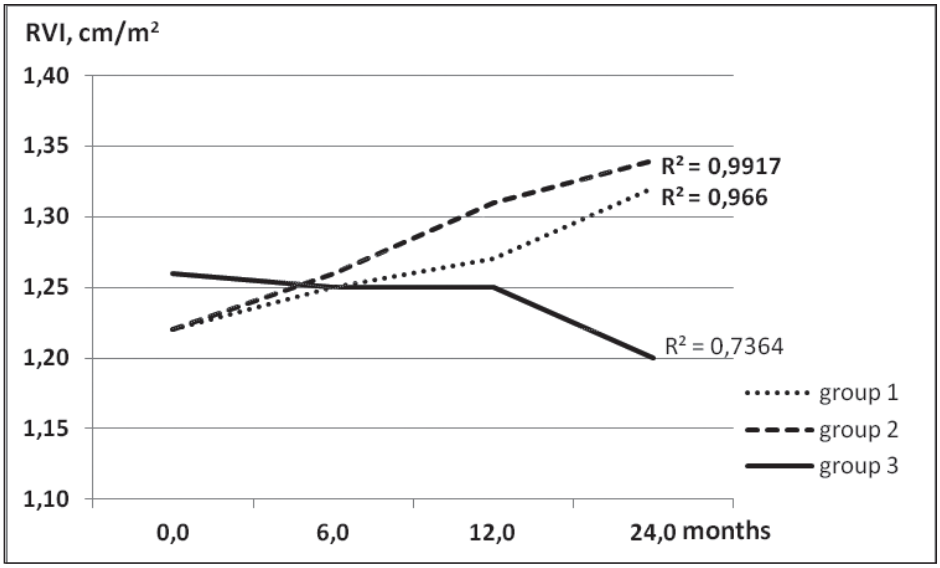

Fig. 4. Serial changes of left atrial, left and right ventricular dimensions during follow-up. LA = left atrial antero-posterior dimension, LV EDDI = left ventricular end-diastolic dimension index, RVI = right ventricular index.

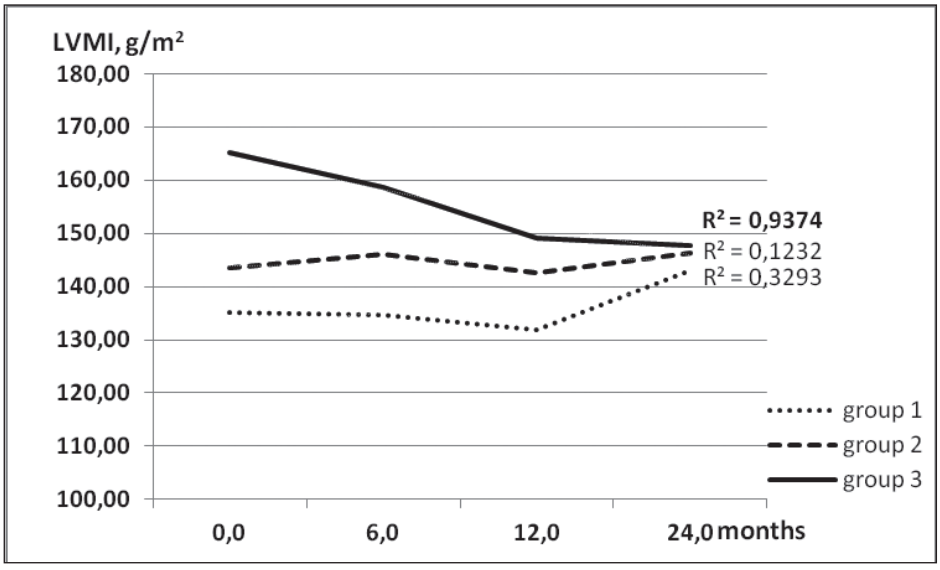

Fig. 5. Serial changes left ventricular mass index (LVMI) and relative wall thickness (RWT) during follow-up.

Positive changes of mass indexes were also seen, i.e., left ventricular mass index and relative wall thickness significantly decreased (Figure. 5, 6).

Left ventricular function was improving in the ramipril and zofenopril groups (Figure. 7, 8) as shown by serial changes of LV ejection fraction and fractional shortening. Thirty-day

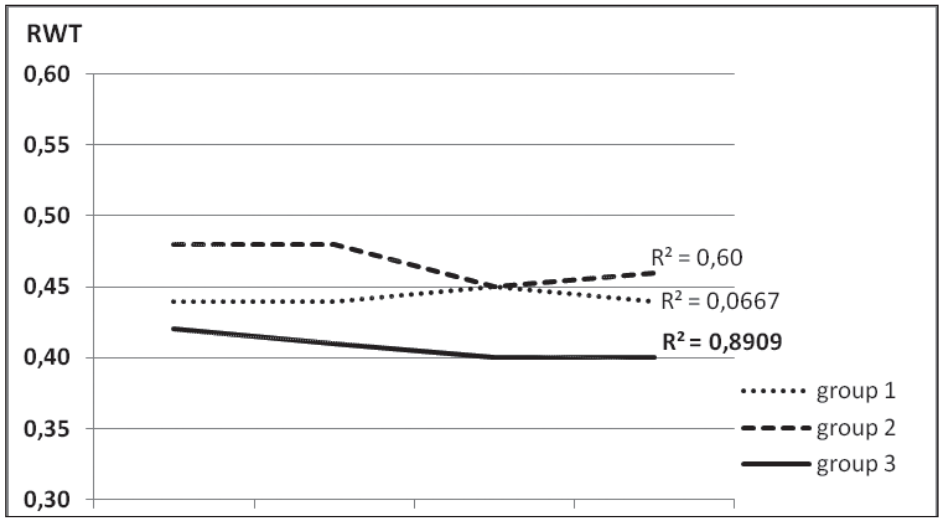

Fig. 6. Serial changes left ventricular mass index (LVMI) and relative wall thickness (RWT) during follow-up.

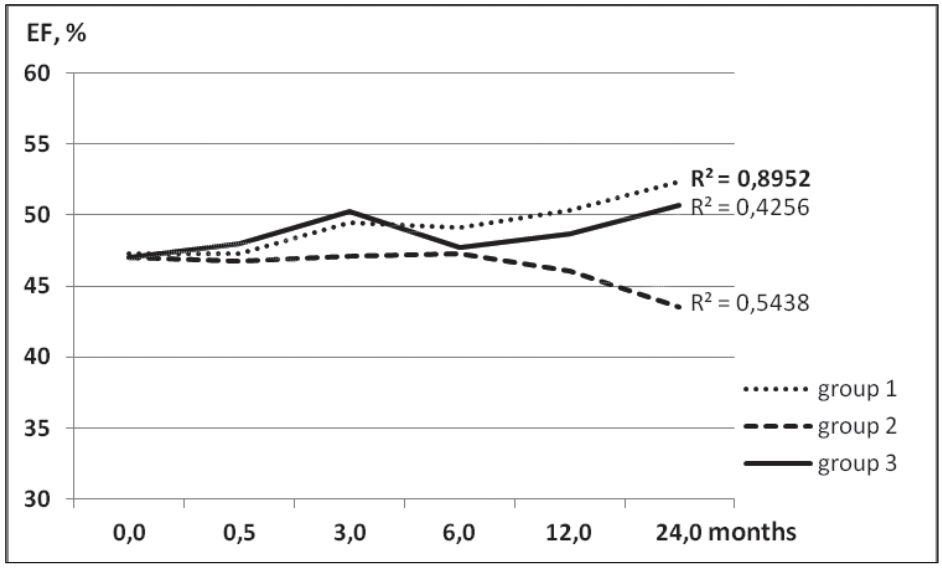

Fig. 7. Serial changes left ventricular ejection fraction (EF) and fractional shortening (FS) during follow-up.

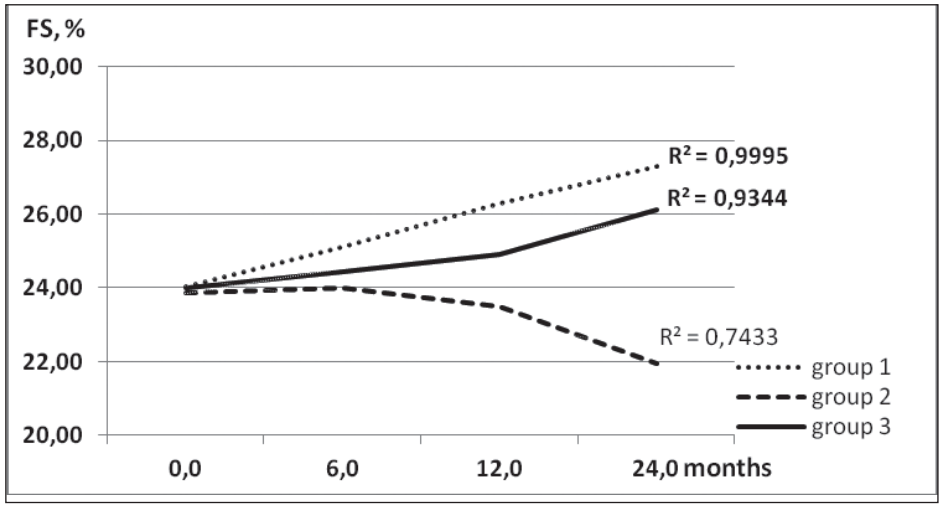

Fig. 8. Serial changes left ventricular ejection fraction (EF) and fractional shortening (FS) during follow-up.

mortality in groups 1-3 was significantly different in the ramipril and perindopril groups (p=0,003) as compared with zofenopril group. No 30-day mortality was registered in zofenopril group. There was no statistically significant difference between the groups in terms of causes of death. Lower survival in perindopril group (group 2) was possibly related to age factor (Table III).

DISCUSSION

As the baseline clinical, morphometric and functional characteristics of patients of all three groups who were found to be compliant with the prescribed therapy were similar, this allowed to assess the effectiveness of different ACEI in the secondary prevention of MI.

Table III. Mortality rates

Variables	Group 1, n=36	Group 2, n=21	Group 3, n=19	P value
30-day mortality,%	4.7	12.9	3.8	p1-3= 0,778 p1-2= 0,003 p2-3= 0,063
One-year mortality*, %	10.3	23.4	5.8	p1-3= 0,317 p1-2= 0,0004 p2-3 = 0,005

*Log-rank p value between the groups is 0,01

Our observations support the assumption that the effect of various ACEI on postinfarction myocardial remodeling may be different. In contrast to ramipril and perindopril which were associated with a transient increase of NT-proBNP levels, zofenopril was associated with consistent lowering of NT-pro BNP throughout the follow-up. Thus, the observed serial changes of NT-proBNP levels indicate that zofenopril, as compared with ramipril and perindopril, may be considered the preferred agent for prevention of post-MI heart failure.

Different ACEI were also associated with different tendencies in terms of postinfarction myocardial remodeling. As compared with ramipril and perindopril, zofenopril showed a more pronounced effect on prevention of post-MI LV dilatation as well as a favourable effect on LV mass indexes.

The effect on LV contractility as assessed by serial changes of LV ejection fraction and fractional shortening was favourable in all three groups. However, these findings should be interpreted with caution. While analyzing these changes in context with concomitant morphological changes (i.e., increase of ventricular dimensions in the ramipril group) one may speculate that the increase of the LV ejection fraction occurred according to Frank-Starling law, thus putting these patients at risk of further LV dilatation and development of ischemic cardiomyopathy.

Also, different survival rates illustrate the differences between the effects of the studied ACEI. The use of zofenopril was shown to be associated with better survival.

CONCLUSION

Zofenopril may have advantages in the long-term complex management of post-MI patients over perindopril and ramipril in terms of prevention of negative myocardial remodeling, onset of congestive heart failure and major adverse events.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

DISCRETE LABORATIRY AND MORPHOMETRIC MARKERS OF ATHEROSCLEROTIC LISIONS OF LOWER EXTREMITY VESSELS

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ABSTRACT

The aim: To investigate discrete laboratory and morphometric features of atherosclerotic lesions in patients with chronic ischemia of the lower extremities (CILE).

Materials and methods: The examined contingent consisted of 47 patients (age 56.32 ± 1.09 years) diagnosed with obliterating atherosclerosis of the lower extremities. The study included determination of levels of Homocysteine, Folic acid, C protein, quantitative determination of circulating Endothelial cells (DEC) in blood plasma, and morphometric study of DEC.

Results: Protein C levels are within the reference values (0.97 ± 0.12 mg / l. In 37 (78.7%) patients Hypoacidofoliemia (<3.0 mmol / l) was observed. Homocysteine levels were clearly elevated in all patients. In the vast majority Hyperhomocysteinemia mild form (91.5%) was observed. The number of DEC in patients was -3.22 ± 0.39x10^5/l and after compression –6.12 ± 0.21x10^5/l

Conclusions: Protein C levels were within the reference values (0.97 ± 0.12 mg / l); Folic acid levels in the vast majority (37 patients, 78.7%) were <3.0 mmol /l. Blood plasma Homocysteine levels were clearly elevated in all patients. The mild form of Hyperhomocysteinemia (91.5%). was observed in the vast majority The number of DEC in patients was -3.22 ± 0.39x10^5 and after compression –6.12 ± 0.21x10^5/l, which confirmed the presence of the endothelial dysfunction.

KEY WORDS: patients, obliterating atherosclerosis of lower extremity vessels, endothelium, hyperhomocysteinemia, morphometry

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INTRODUCTION

The attention of scientists is focused on the direction of endothelium-targeted research, as the earliest target organ and the key link in disease pathogenesis, according to literary sources, in our time [1]. The endothelium is an active endocrine organ, one of the largest in the body, diffusely located intravascularly across all tissues. The endothelium, which is distributed throughout the body, is essentially an organ with a huge surface.It is a differentiated complex structure which has specific functions in the body. It is a part and a control object of one of the organism's systems – of the circulatory system. By histologists' definition, the endothelium is a single-layer of specialized cells lining the entire cardiovascular tree from the inside, weighing about 1.8 kg. [2]. The blood capillaries, which are the ultimate executive link in the implementation of the transport functions described, are sometimes referred to as blood vessels. They complete one of the main functions of the circulatory system – the bilateral metabolism between the blood and the interstitial fluid of body tissues. From the microcirculatory vascular system of blood into the interstitial fluid comes the substances necessary for tissue metabolism, and from the interstitial fluid into the blood are transported the final products of tissue metabolism. The efficiency of such two-way transport of substances is ensured by the considerable duration, time of contact of blood with a huge surface of exchange of microcirculatory channel. This surface is formed by endothelial cells, whose total surface area exceeds ≈ 1000 m2[1,2].

An important pathogenetic link in the development of atherosclerotic vascular damage is the homocysteine theory. The development of atherosclerosis explains the possible connection of hyperhomocysteinemia (HHC) with the formation of vascular pathology on the basis of this theory, the fasting homocysteine concentration of more than 12.1 μmol / l is associated with a 2-times increase in the risk of atherosclerosis, including obliterating atherosclerosis of the lower extremities (OALE), coronary heart disease (coronary artery disease), acute coronary artery disease regardless of other risk factors, according to European studies. A study of the role of Homocysteine (HC) in the development of atherosclerosis revealed that the correlation coefficient between Coronary artery disease and cerebrovascular accident is 0.5-1.0 for every 5 μmol / l increase in Homocysteine, according to a meta-analysis. Approximately 30-40% of patients with OALE have elevated levels of Homocysteine. HHC is thought to increase the risk of progression of OALE, but the etiological role of HHCs remains unknown, as no studies have been reported to study the reduction of HCC levels in OASNCs. Much of the HC is remethylated with the formation of methionine. Folic acid and its derivatives are the main source of tetrahydrofolate, in which deficiency can develop HHC. At this point, other potential mechanisms for the emergence of endothelial dysfunction and its defeat at HHC have become known. Circulating desquamated endothelial cells are cells that separate from the vessel wall in the course of its damage

Table I. Laboratory markers of vascular atherosclerosis development (n=47)

Parameters	Number of leukocytes (x10⁹/л)	Protein C screening (0,8-1,15мg/l)	Folic acid (3,0-17,0 mmol/l)	Homocysteine (5,0-12,0 mmol/l)
M±m	8,2±0,3 (5-14)	0,97±0,12 (1,26-0,63)	2,22±0,37 (6,71-1,22)	18,29±1,18 (40,20-11,50)

and are a direct cellular marker of endothelial dysfunction [1, 2]. The concentration of desquamated endothelial cells in the peripheral blood is very low in the in the physiological state, since the endothelial self-renewal process is slow and the desquamated cells are intensively absorbed in the bloodstream by the macrophage system [3]. At the same time, there were increase in the number of these cells in various pathological conditions associated with the defeat of the vascular system (vasculitis, cardiovascular disease etc.) [1, 3]. It can be argued that the level of desquamated endothelial cells in the peripheral blood is a reflection of systemic damage of the endothelium based on this fact [4,5].

THE AIM

To investigate discrete laboratory and morphometric features of atherosclerotic lesions in patients with chronic ischemia of the lower extremities (CILE).

MATERIALS AND METHODS

The study was conducted on the basis of the ZRKH, named after Andrew Novak, Uzhhorod. The examined contingent consisted of 47 patients (age 56.32 ± 1.09 years) diagnosed with obliterating atherosclerosis of the lower extremities. The study included determination of levels of Homocysteine, Folic acid, C protein, quantitative determination of circulating Endothelial cells in blood plasma, and morphometric study of Endothelial cells. The study of HC levels in the blood plasma of patients was performed by enzyme-linked immunosorbent assay (ELISA) with using the test of Axis-Shield (UK) kit. The concentration of Folic acid in the serum of patients was investigated using an immunochemical method with electrochemiluminescent detection on ACL TOP 700 coagulometer, manufactured by Instrumentation Laboratory, USA. For the study of protein C the test systems of reagents manufacturer by "Roche Diagnostics" (Switzerland) was used. "A set of reagents for screening assessment of disorders in the system of protein C (Protein C-screening test) according to TU 9398-276-05595541-2009"was used. All patients underwent venous blood sampling, followed by determination of the number of desquamated endothelial cells (taking into account morphometric characteristics), which determined the degree of endothelial dysfunction. For the diagnosis of endothelial dysfunction in patients in the shoulder region create a positive pressure in excess of systolic blood pressure by 40-50 mm Hg, after 4 minutes carry out decompression, followed by sampling of venous blood with subsequent determination of the number of desquamated endothelial cells by Hladovec J. in terms of 1 liter of plasma [6]. Endothelial damage was assessed in the presence of desquamated endothelial cells (DEK) in the blood plasma of patients at a value greater than 2.77×10^5 /l of plasma. The method has a high sensitivity and allows to diagnose latent endothelial dysfunction (patent RU (11) 2234094 (13) C2). MicrosMCX-100 Daffodil microscope was used for the work[1]

RESULTS

The first stage of the study was the laboratory search for disorders that contribute to the development of vascular atherosclerosis and clinical presentation in patients with CILE. Disorders of Homocysteine amino acid metabolism and interdependent markers of folic acid and protein C were investigated (Table 1)

Protein C levels are within the reference values (0.97 ± 0.12 mg / l) according to table I. In 5 patients, from the experimental group, the values (<0.8 mg / l) were decreased, in 4 patients -> 1,5 mg / l. Protein C is an indicator of the blood coagulation system, one of the major inhibitors of the coagulation process and is one of the most important components of the anticoagulant blood system. The data obtained indicate the controlled state of the hemostasis in patients. The following picture was observed in the study of folic acid levels. In 37 patients, hypoacidofoliemia (<3.0 mmol / l) was observed, accounting for 78.7% of the total number of patients. Increasing Folic acid was not fixed.

When assessing the degree of homocysteine, blood plasma has the following gradation of abnormalities: mild HHC (10–30 μmol / l), moderate HHC (30–100 mmol / l) [7] Homocysteine levels were clearly elevated in all patients. In 4 patients a mild form of hyperhomocysteinemia (8.5%) was observed, in the vast majority was a mild form of HHC (91.5%). Studies over the past 15 years have found that homocysteine is a ranked independent risk factor for cardiovascular disease (CVD) – myocardial infarction, stroke, and venous thromboembolism, atherosclerosis [7,8]. HHC is a more informative indicator of the development of diseases of the cardiovascular system than cholesterol according to scientists data.

Homocysteine significantly reduces protein C activation by competitively inhibiting of the thrombo-modulin thrombin interaction required for thrombin S. protein activation. Homocysteine is also capable of interfering with the regulation of fibrinolysis. Vascular endothelium regulates local processes of hemostasis, proliferation, cell migration into the vascular wall and vascular tone. The idea of endothelial dysfunction was formed, which means the imbalance between the factors that provide all these processes. Fixation of endothelial cells on the basement membrane is carried out with the help of vitronectin, fibronectin, cadherins and more effective in young cells. The process of desquamation reflects the renewal of the endothelium, which has lost its ability to perform its inherent function as a result of aging or the effects of harmful factors. The endothelium desquamation is based on the activation of proteinases, necrosis and / or apoptosis of endothelial cells.

Table II. Counting of DEC in patients (n = 30) with obliterating atherosclerosis of the lower extremities

Parameters	Age (years)	Number DEC before probe	Number DEC after probe	P
	56,32±1,09	3,22±0,39x105	6,12±0,21x105	<0,001

In the case of injury and apoptosis, there is a disruption of the functioning of proteins that provide the connection of endothelial cells with the basement membrane, which leads to desquamation of endothelial cells. The duration of finding of circulated endothelial cells in the blood is about 24-42 hours, during which their capture and destruction by macrophages of the liver, lungs and spleen occurs. Apoptosis and necrosis of endothelial cells, increased production of proteinases, breaking the endothelial cell junction with underlying intimacy, promote the release of cytokines, free radicals and reactive oxygen species. The source of these biologically active substances may be leukocytes, especially those endogenous to the endothelial cells, which confirm the body's inflammatory response. Risk factors of atherosclerosis along with cardiovascular factors are infectious agents: Chlamydophilia pneumonia, symptoms of viral herpes, Helicobacter pylori and cytomegalovirus, which enhance the procoagulant properties of the endothelium. During the chronic inflammatory reaction, migration and proliferation of smooth muscle cells occur, which contributes to the further progression of atherosclerosis [9]. The endothelium is characterized by high stability, which is confirmed by the rare detection of apoptotic endothelial cells in the intima of vessels in normal state, in some other pathological processes, reflects the degree of damage of the vessels and allows to judge the severity of the disease, the effectiveness of the therapy. Adhesion of circulating leukocytes to the wall of blood vessels, as well as migration into the subendothelial space is carried out by adhesion molecules. During proteolytic cleavage, soluble forms of adhesion molecules appear, a sensitive indicator of the extent of atherosclerotic lesions of the arteries [10]. The table presents the quantitative characteristics of the level of desquamated plasma endothelial cells in patients before and after the test with compression of the vessels of the shoulder (Table II).

At the start of the study, the number of desquamated endothelial cells in patients exceeded the reference values (3.22 ± 0.39x105) and after of compression the DEC amount increased in 2-times (6.12 ± 0.21x105/1in of plasma). The high level of DEC in patients confirmed the presence of the endothelial dysfunction in the patients. A significant increasing in more than one percent of the DEC level in the blood after vessels compression of the shoulder vessels indicates their tendency to desquamation with minor mechanical effects (external – compression; internal – shear stress after decompression) and testifies to endothelial dysfunction. Based on the studies, it should be considered that the increase in the number of circulating desquamated endothelial cells above 2.77x105/l of plasma after a sample with short-term compression of the blood vessels is a diagnostic criterion of endothelial dysfunction, which was confirmed in our investigation. Based on a set of general clinical and special research methods, patients in the study group confirmed the presence of endothelial dysfunction. We also conducted a morphometric study of endothelial cells in the blood of patients with chronic lower extremity ischemia (Figure 1)

In the case of injury and apoptosis, there is a malfunction of proteins that provide endothelial cell connections with the basement membrane, which leads to endothelial cell desquamation. The source of these biologically active substances may be leukocytes.

DISCUSSION

Hyperhomocysteinemia damages the walls of the vessels, making their surface loose. Cholesterol and calcium precipitate, forming atherosclerotic plaques on the damaged surface. Increased levels of Homocysteineemia increase thrombosis. Inhibiting the work of the anti-coagulation system, Hyperhomocysteinemia is one of the links in the pathogenesis of early thrombovascular disease, as it increases the risk of vascular thrombosis. Homocysteine is also capable of interfering with the regulation of fibrinolysis. The Homocysteine detrimental effect on vascular endothelium is realized through a direct damaging effect on the blood vessels by auto-oxidation products, enhanced low-density lipoprotein peroxidation, and inhibition of endothelial cells DNA synthesis . The multifactorial genesis of atherosclerosis and thrombosis is undeniable. The authors, at the same time, who deny the significant role of HHC in the pathogenesis of cardiovascular disease, do not take into account the presence of other proven or actively investigated proatrogenic or prothrombotic determinants. One of these factors is HHC, which is an important determinant in the development of thromboembolic complications and endothelial dysfunction in various diseases and conditions. Adhesion of circulating leukocytes to the wall of blood vessels is carried out by adhesion molecules, as well as migration into the subendothelial space Examination for the detection of HHC is advisable to perform in patients with vascular pathology. Significant increase in the greater percentage of cases of the amount of DEC in the blood of patients after shoulder vessels compression indicates their tendency to desquamation with minor mechanical effects (external – compression; internal – shear stress after decompression) and indicates endothelial dysfunction. Based on the studies, it should be considered that the increase in the number of DEC circulating ($6.12 \pm 0{,}21x10^5$/l) in plasma after short-term compression of the shoulder vessels is a diagnostic endothelial dysfunction criterion, which was confirmed in ours. The presence of endothelial dysfunction, Hyperhomocysteinemia as determinants of the development of the lower extremities vessels atherosclerotic lesions was confirmed in the patients of the study group, on the basis of the complex of laboratory and morphometric methods of investigation.

CONCLUSIONS

1. The level of Protein C is within the reference values (0.97 ± 0.12 mg / l)

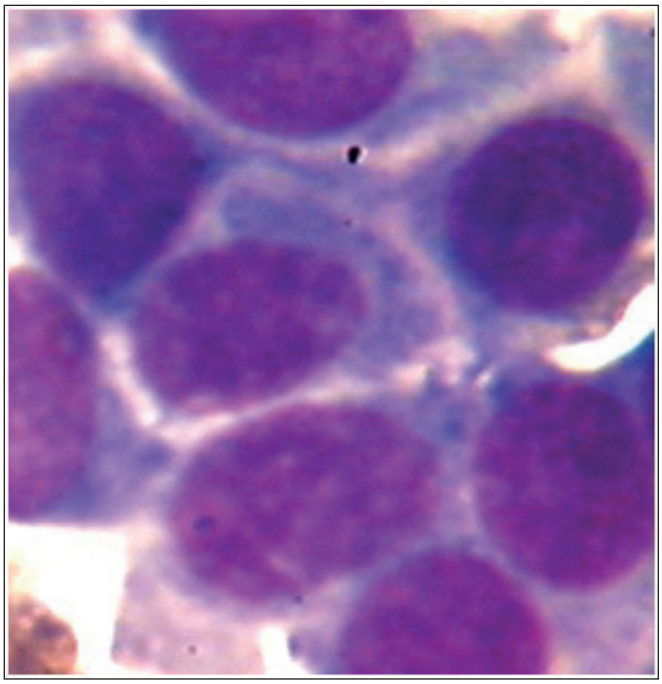

Figure 1. Endothelial cell layer: pronounced polymorphism and proliferation, nucleoli are visualized, coarse-grained chromatin, the nucleus occupies almost all the cytoplasm.

2. Hypoacidofoliaemia (<3.0 mmol / l) was observed in 37 patients in the study of folic acid levels, representing 78.7% of the total patients number. An increasing in folic acid levels has not been recorded.
3. Plasma Homocysteine levels were clearly increased in all patients. In 4 patients a mild form of Hyperhomocysteinemia (8.5%) was observed, in the vast majority was a Hyperhomocysteinemia mild form (91.5%).
4. The number of desquamated blood plasma endothelial cells in patients exceeded the reference values (3.22 ± 0.39x105) and the amount of DEC increased in 2-times(6.12 ± 0.21x105/l) of plasma after compression, which confirmed the presence of the investigated endothelial dysfunction.
5. At morphometric research of the endothelial cells layer expressed polymorphism and proliferation is observed; nucleoli, coarse-grained chromatin are visualized, the nucleus occupies almost all cytoplasm.

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THE HISTOTOPOGRAPHIC FEATURES OF FORMATION OF KELOID SCARS OF MAXILLOFACIAL LOCALIZATION

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ABSTRACT

The aim: Study of the influence of local oxygen deficiency on the features of the formation of keloid scars of the head and neck.

Materials and methods: The research material was 17 incisional biopsy specimens of keloid scars, which were the highest age categories of patients from 19 to 63 years.

Results: It was revealed that at coloring according to Mallory's technique, sections of fibrinoid swelling of collagen fibers were noted near central homogeneous hyaline masses. The lateral areas of the dermis that adjacent to keloid scar tissue, at coloring by the Hart method, in our modification, were characterized by the separation of the hyalinosis zone from the undamaged dermis by coarse bundles of elastic fibers that are colored in dark purple. The undifferentiated dysplasia of the connective tissue has caused the replacement and obstruction of single vascular components, which has complicated the local oxygen deficiency of keloid-altered tissues.

Conclusions: Thus, plasmaragia and the accumulation of protein deposits in the perivascular space determine the inhibition of local hemodynamics, which explains the decrease in oxygen transport to tissues. Decreased oxygenation and increased permeability of the vascular wall causes local hemocirculatory hypoxia.

KEY WORDS: keloid scar, hyalinosis, histopographic features

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INTRODUCTION

Scars are visible and palpable skin changes that remain after healing various types of damage to the integrity of the skin. Scarring is a pathophysiological process of skin regeneration, which is directed at closing of defect. Atypical wound healing may be accompanied by excessive scarring. In this case, randomly (chaotically) located fibers of dense connective tissue form hypertrophic or keloid scars. The regulation of process of scar formation depends on many iatrogenic and somatic factors. The problem of excessive scar formation deserves a particular attention, primarily in the open areas of the human body [1-4].

Long-term treatment of wounds, purulent inflammatory processes, the presence of various pyogenic microorganisms in them, as well as a weakening of the reactivity and resistance of the body, including tissue immunity, lead to atypical development of connective tissue with the formation of an altered musculoskeletal structure – scars. They cause not only cosmetic, but also physiological disturbances [5-8].

Therefore, the problem of differential diagnosis and comprehensive rehabilitation treatment of head and neck scars remains an urgent problem of modern medicine.

THE AIM

Study of the influence of local oxygen deficiency on the features of the formation of keloid scars of the head and neck.

MATERIALS AND METHODS

The research material was 17 incisional biopsy specimens of keloid scars of different topographo-anatomical areas of maxillofacial localization, which were the highest age categories of patients from 19 to 63 years.

To achieve the established goals, the studied tissues were stained using the Mallory method, Hart + Van Gieson (in our modifications) and amydo-black (in our modifications) [9-11]. A digital camera was used for systematic documentation.

RESULTS AND DISCUSSION

Based on the literature, a visually keloid scar is characterized by a protruding part of the skin covered with the epidermis, under which there are hyaline masses [12, 13].

The histochemical studies were performed to determine the histochemical properties of fibrous structures of keloid scar. It was revealed that at coloring according to Mallory's technique, sections of fibrinoid swelling of collagen fibers were noted near central homogeneous hyaline masses. The latter were almost completely destroyed with the formation of fiber-fibrous structural elements, and in some areas the fibers and structural structures of the fibers were represented (Figure. 1).

The lateral areas of the dermis that adjacent to keloid scar tissue, at coloring by the Hart method, in our modification,

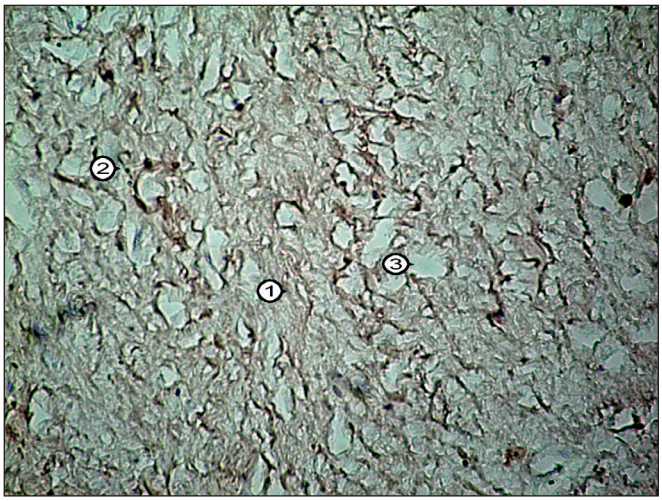

Fig. 1. Plots of fibrinoid swelling of keloid scar. Colored by Mallory. Ob .: x 40: Ok .: x 10.
1-fragmented collagen fibers; 2-protein composites; 3-regressed vessels.

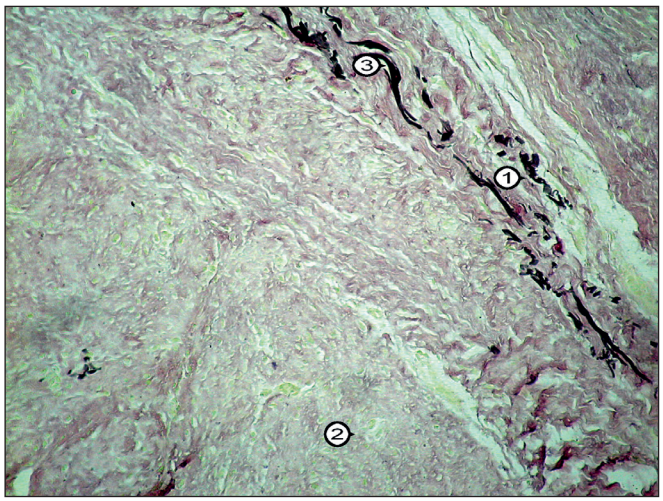

Fig. 2. Areas of the dermis that border to keloid scars. Colored by Hart (in our modification). Ob: x 20: Ok .: x 10:
1-intact dermis; 2-zone of hyalinosis; 3-elastic fibers.

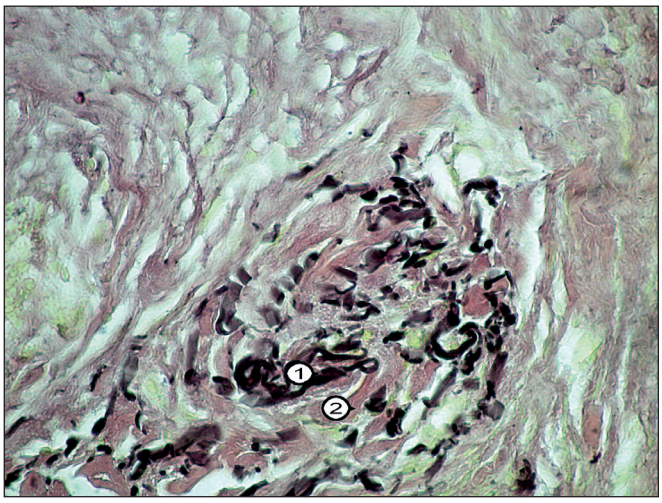

Fig. 3. The formation of a neuroma in the main zone of keloid scars. Colored by Hart (in our modification). Ob.: x 100: Ok .: x 10:
1-neuroma; 2-collagen fibers.

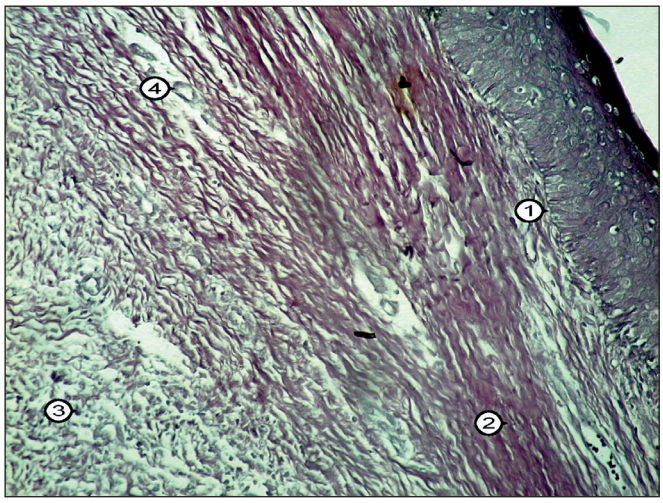

Fig. 4. Change of tinctorial properties of collagen fibers. Colored by amido-black (in our modification). Ob .: 40: Ok .: x 10.
1-basal membrane; 2-tinctorially modified collagen fibers; 3-core zone; 4-vascular components.

were characterized by the separation of the hyalinosis zone from the undamaged dermis by coarse bundles of elastic fibers that are colored in dark purple.

Hyperelastosis, that was observed in the lateral areas of the keloid scar, in our opinion, should be regarded as an adaptive-compensatory process for formation of hyalinosis.

It has been established that the basis of the keloid scar reaches the borders of dermis, which consists of coarse bundles of collagen fibers were painted in red color. These bundles were separated from each other by bundles of elastic fibers that were painted in dark purple.

Between the aforementioned fibrous structures it was noted the vascular bundle, which was represented by arteries and veins. In the arteries, the external and internal membranes were clearly defined, painted in purple.

The circular smooth muscle layer was located between the membranes, which was colored green. Unlike arteries, bundles of collagen and elastic fibers in the veins were intertwined.

It is obvious that precisely due to the presence of a vascular bundle in the basis of the keloid; a compensatory-adaptation process in the form of hyperelastolysis is carried out around it. The undifferentiated dysplasia of the connective tissue has caused the replacement and obstruction of single vascular components, which has complicated the local oxygen deficiency of keloid-altered tissues (Figure. 2).

It should be noted that in some cases the neuromas were visualized on serial histological sections at the base of the keloid scar at stained according to the Hart method, in our modification. The latter were represented by concentric, spiral-like myelin fibers, which were colored black. Between them bundles of collagen fibers colored in red were noted. In our opinion, the partial reparative regeneration of myelin fibers occurs is happened during the growth

of the latter. Obviously, the presence of a neuroma in the thickness of the keloid scar falling under compression of collagen and elastic fibers causes the subjective sensations of patients (local pain, paresthesia and itching) (Figure. 3).

It was found that at coloring by amido-black, in our modification, in the areas of keloid scars adjacent to the epithelium, the papillary layer of the dermis was replaced by individual bundles of collagen fibers. The structure of the epidermis was preserved with the presence of horny scales.

Separate bundles of collagen fibers have stacked together and gradually have replaced the papillary layer, which included arcade microvessels. There were single cell infiltrations between bundles of collagen fibers. It was established that the keloid scar in case of this technique of coloring have consisted of thin fibrillar structures.

The bundles of fibrous structures during histological examination at coloring by amido-black were colored red due to changes in their tinctorial properties. In our opinion, a change in these properties when applying the above-mentioned color technique was due to the phenomenon of fibrinoid swelling.

Fibrinoid swelling, which was observed in keloid scars, has indicated its constant progression in the chronic course in the presence of various exogenous factors. Between the individual arteries and venules, there were light homogeneous structures of the protein deposit, among which there were single elongated fibroblast nuclei (Figure. 4).

Therefore, at the first stage of the formation of a keloid scar, a gradual replacement of the papillary dermis with bunches of collagen fibers, which were formed in case of presence of cellular infiltrates, was observed.

The second stage of keloid morphogenesis was characterized by fibrinoid swelling and necrosis of collagen fibers.

It should be noted that in conditions of progression of the keloid scar at its base, along with the phenomena of fibrinoid swelling of collagen fibers, plasma hemorrhage from the vessels was noted. The connective tissue hyalinosis was observed due to fibrinoid swelling and plasmoragia from blood vessels in the central zone of the keloid scar.

CONCLUSIONS

Thus, plasmaragia and the accumulation of protein deposits in the perivascular space determine the inhibition of local hemodynamics, which explains the decrease in oxygen transport to tissues. Decreased oxygenation and increased permeability of the vascular wall causes local hemocirculatory hypoxia. Therefore, in our opinion, to eliminate the local oil-deficient state, emoxipin should be used, which stabilizes the vascular wall and reduces tissue hypoxia by suppressing lipid peroxidation processes.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

INDICATORS OF INFLAMMATION IN THE PATHOGENESIS OF UNSTABLE ANGINA

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ABSTRACT

The aim: Research of blood lipid spectrum, level of anti-inflammatory cytokines and C-reactive protein of coronary heart disease patients.

Materials and methods: There was examined 61 patients with unstable angina, who had been on hospital care in the cardiology department of the Lviv National Emergency Hospital. Their average age was 68.3 ± 1.9 years. The control group included 20 generally healthy persons. There was estimated blood lipid spectrum, C-reactive protein, fibrinogen and proinflammatory cytokine of patients.

Results: There was determined considerable increase total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, triglycerides and coefficient of atherogenicity. High level of C-reactive protein and pro-inflammatory cytokines were detected in patients with unstable angina.

Conclusions: In patients with unstable angina was revealed a significant increase of proinflammatory cytokines levels in the blood serum: interleukin-1β, interleukin-6, interleukin-17, TNF-α and C-reactive protein, fibrinogen, which indicates activation of the inflammatory process. In patients with unstable angina was detected a significant disorder of blood lipid spectrum. For its correction should be recommended diet and hypolipidemic agents.

KEY WORDS: unstable angina, interleukins, C-reactive protein

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INTRODUCTION

Cardiovascular disease is a leading cause of population disability and mortality worldwide, and angina is the most common symptom of cardiovascular disease [1, 2, 3, 4, 5]. At last count, approximately 9 million people in the United States have coronary heart disease [4]. For the last two decades, ischemic heart disease mortality in Poland increased in people under 65 [3]. Thus, cardiovascular disease is an important medical, biological and social problem as in Ukraine as in other countries of the world. That is why improving diagnostics and strategies for therapy is essential and priority [3, 5, 6]. Pathophysiological basis for the development of cardiovascular diseases, namely coronary heart disease (CHD) is coronary atherosclerosis. The morphological substrate of atherosclerosis is an atherosclerotic plaque, which prevents perfusion in the affected segment, contributes to reduction of coronary blood flow and leads to myocardial ischemia [3, 7].

Significantly increases risk of atherosclerosis at CHD in availability of related risk factors: hypertension, dyslipidemia, smoking, being male, old age, diabetes, increased heart rate, low physical activity, excess body weight, climatic and geographical factors and others. Nowadays, they number more than 200. Though, smoking 1.6 times more increases the risk of coronary complications, hypertension (systolic arterial vise greater than 195 mmHg) – 3 times, hypercholesterolemia (8.5 mmol / l, 330 mg / dcl or more) – 4 times, the set of three risk factors – 16 times [8, 9].

Unstable angina (UA) constitutes acute coronary syndrome [10, 11]. In unstable angina, the duration of ischemia is insufficient to cause myocardial necrosis. Based on current data, the trigger mechanism of the pathogenesis of unstable angina is damage to the atherosclerotic plaque with thrombosis and episodes of spasm with subsequent progression of stenosis. [3, 7, 12, 13, 14]. An atherosclerotic plaque is formed from an atheromatous (lipid) core and fibroids, which includes smooth muscle cells, collagen, proteoglycans, macrophages, lymphocytes [15]. When an unstable plaque is damaged, after the rupture or erosion of the atherosclerotic plaque, coagulation factors interact with the thrombogenic content of the plaque, specifically collagen, thrombosis are developing. If the balance between prothrombotic and fibrinolytic mechanisms is unfavorable – occlusive blood clots are formed in this section of the vessel crease, which cause acute coronary syndromes, specifically unstable angina. The plaque vulnerability depends on the location, size and

Table I. Risk factors of coronary heart disease in patients with unstable angina

Risk factors	Total patients with UA (n=61)		Male (n=22)		Female (n=39)	
	абс.	%	абс.	%	абс.	%
Age	57	93,4	21	95,5	36	92,3
Heredity	14	22,3	4	19,0	10	25,6
Arterial Hypertension	57	93,4	20	90,9	37	94,9
Hypodynamia	28	45,9	10	45,5	18	46,2
Overweight	12	19,7	5	22,7	7	17,9
Impaired glucose tolerance	12	19,7	3	13,6	9	23,1
Obesity	17	27,9	3	13,6	14	35,9
Dyslipidemia	49	80,3	18	81,8	31	79,5
Smoking	16	26,2	15	68,2	1	2,3
Alcohol consumption	5	8,2	4	18,2	1	2,3
Psycho-emotional factors	21	34,4	7	31,8	14	35,9
Occupational hazards	8	13,1	4	18,2	4	10,3

Table II. Clinical characteristics of patients with unstable angina

Risk factors	Total patients with UA (n=61)		Male (n=22)		Female (n=39)	
	абс.	%	абс.	%	абс.	%
Nature of pain						
Clutching	53	87,0	19	86,4	34	87,2
Scorching	8	13,0	3	13,6	5	12,8
Localization of pain						
Behind the sternum	57	93,4	20	90,9	37	94,9
In the heart area	4	6,6	2	9,1	2	5,1
Irradiation	11	18,0	3	13,6	8	20,5
SBP mm Hg	153,2	-	150,7	-	153,2	-
DBP mm Hg	90,4	-	89,1	-	91,2	-
HR beats / min	84,0	-	88,2	-	102,9	-
Irregular heartbeat	21	31,8	10	45,5	11	28,2
Conduction abnormality	4	6,6	3	13,6	1	2,6
Ejection fraction, %	61,3	-	60,5	-	61,8	-

composition of the lipid nucleus, circular wall tension, the influence of blood flow on the surface of the atherosclerotic plaque (sher stress) [10, 11, 14, 15, 16]. If the balance is favorable, the blood clot is absorbed, at the site of the lesion there is a growth of connective tissue and calcification of the vascular wall, which, in turn, leads to deformation and stenosis of the vascular gap with consequential emergence of symptoms of stable angina. [16]. It should be noted that the process of destabilizing the fibrous coating of the plaque, especially its rupture, is accompanied by activation of local inflammation. Pathomorphological researches indicate the presence of a large number of inflammatory cells, T-lymphocytes, monocytic macrophages on the place of rapture or on the surface of plaque erosion [9, 13, 17].

Endothelial dysfunction has a great role in the process of atherogenesis. It is an important cause of insufficient vasodilation and spasm of coronary arteries of different caliber [9].

To understand the processes that develop in an atherosclerotic altered vascular wall, it is important to study the markers of inflammation, since inflammatory processes play an important role in the formation and destabilization of atherosclerotic plaque. From inflammatory markers attract a much attention C-reactive protein (CRP), proinflammatory cytokines: interleukin-1β (IL-1β) interleukin-6 (IL-6), interleukin-17 (IL-17), necrosis factor of alpha tumors (TNF-alpha) [18, 19, 20, 21].

THE AIM

Investigation of blood lipid spectrum, levels of proinflammatory cytokines and C-reactive protein, fibrinogen in patients with coronary heart disease.

Table III. Indicators of blood lipid spectrum in patients with unstable angina and control group (M±SD)

Indicators	Patients with UA (n=61)	Control group (n=20)
Total CS, mmol / l	6,02±1,14*	4,77±0,98
HDL, mmol / l	1,21±0,27	1,33±0,48
TG, mmol / l	1,96±0,92*	1,30±0,44
LDL, mmol / l	3,91±0,96*	2,85±0,99
VLDL, mmol / l	0,89±0,42*	0,59±0,20
IA	3,95±1,45*	2,59±0,97

* - The credibility of the difference between the indicators of the control group (p<0,01)

Table IV. Indicators of inflammation in patients with unstable angina and control group (M±SD)

Indicators	Patients with UA (n=61)	Control group (n=20)
CRP mg / l	9,81±4,12*	3,92±1,55
IL-1β, pg / ml	5,90±1,13*	3,20±0,87
IL-6, pg / ml	7,97±2,56*	4,04±1,66
IL-17, pg / ml	15,30±6,02*	8,66±3,11
TNF-α, pg / ml	1,58±0,76*	0,72±0,25
Fibrinogen, g / l	4,47±1,31*	2,86±0,84

* - The credibility of the difference between the indicators of the control group (p<0,01)

MATERIALS AND METHODS

There were examined 61 patients with acute ischemic heart disease, namely with unstable angina (UA), who were hospitalized in the cardiology department of the National Emergency Hospital in Lviv. Among the surveyed were 39 women (64%) and 22 men (36%), their average age was 68,3 ± 1,9 years. The diagnosis of the disease and therapeutic tactics were determined in accordance with the orders of the Ministry of Health of Ukraine, as well as in accordance with the recommendations of the European Society of Cardiologists and the American College of Cardiologists on the management of patients with unstable angina. Patients received standard drug treatment, which included anticoagulants, antiplatelet agents, statins, angiotensin-converting enzyme inhibitors, beta-blockers. To eliminate angina attacks, patients were prescribed nitrates. The study did not include patients with severe cardiac, hepatic, and renal failure, cancer, alcoholism, or drug addiction. The control group included 20 practically healthy individuals of age and gender. The study was approved by the local bioethics committee and conducted in accordance with the principles of the Declaration of Helsinki. The patients were given general clinical and instrumental examination methods, they were determined the indicators of proinflammatory cytokines (interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-17 (IL-17), necrosis factor of alpha tumors (TNF- α)) and C-reactive protein (CRP), fibrinogen. Examination of patients was performed on entry to the hospital.

The lipid spectrum of the blood was determined by an investigation in blood serum of total cholesterol of (STC), triglycerides (TG), high density lipoprotein cholesterol (HDLC) with using reagents from "Human" (Germany). The content of low density lipoprotein cholesterol (LDLC) was calculated using the Friedewald formula: LDLC = STC – HDLC – (TGx0,45), where TGx0,45 – quantity of cholesterol in the composition of very low density lipoproteins (QCCVLDL). The coefficient of atherogenicity (CA) was calculated by the formula A.N. Klimov: IA = STC – QCCVLDL) / QCCVLDL.

Indicators of proinflammatory interleukins were determined in blood serum by enzyme-linked immunosorbent assay using "Vector BEST" test systems (Russia, Novosibirsk) for determination of the content of IL-1β, IL-6, TNF-α, "Cytokine" (Russia, St. Petersburg) – for determination of the IL-17 according to the instructions, which are attached to the reagent kits. Quantitative determination of C-reactive protein in blood serum was performed by immunoturbidimetric method on an automatic Cobas Integra 400 plus analyzer. Fibrinogen was determined by the Claus method.

Statistical processing of the results was performed using Microsoft Exel and Statistica 6.0 by statistical analysis method by Student's t-criterion determination, data are displayed as M ± SD, where M is the arithmetic average, SD is the average deviation, the difference between the groups was considered significant at $p < 0.05$. The correlation between the various factors was estimated using the Spearman rank correlation coefficient. The difference between the groups was considered significant at $p < 0.05$.

RESULTS

By the results of the research among the risk factors for the emergence of coronary heart disease in patients with unstable angina we found: hypertension, dyslipidemia,

hypodynamia, overweight, obesity, smoking, psycho-emotional factors (Table I). Many patients had a combination of several risk factors, which in turn contributes to the progression of angina. The clinical characteristics of patients are presented in the table (Table II).

By the analysis of the data from patients with unstable angina, was revealed disorders of lipid metabolism. In patients with unstable angina, compared with indicators in the control group (Table III), was revealed an increase in total cholesterol, triglycerides, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, atherogenic ratio, before treatment.

Thus, the level of total cholesterol of patients exceeded the value of the control group by 26.2% ($p<0.01$). Triglyceride level significantly exceeded the control figures by 50.1% ($p<0.01$). The level of LDLC in patients significantly exceeded the indicators of the control group by 37.2% ($p<0.01$), accordingly, QCCVLDL – by 50.8% ($p<0.01$). The coefficient of atherogenicity significantly exceeded the control value by 52.5% ($p<0.01$). HDLC in patients was lower compared to the control group. Herewith, HDLC level in patients with unstable angina before treatment less than 1.8 mmol / l (target value for very high-risk patients) was not detected in any patient.

At the analysis of inflammatory markers there was observed their increase compared with the control group (Table IV). In patients with unstable angina pectoris CRP in blood serum significantly exceeded the value of the control group 2.5 times ($p<0.01$). Fibrinogen in patients with unstable angina significantly exceeded the value of the control group 1.6 times. Cytokines of the early acute-phase IL-1β reaction in the serum of patients with unstable angina on entry to hospital significantly exceeded the levels of the control group 1.8 times ($p<0.01$), IL-6, as an indicator of late acute-phase reaction, in serum significantly exceeds the levels of the control group in 2 times ($p<0,01$). Increase of cytokines levels can be considered as a factor that shows the severity of the disease. Interleukin-17 exceeded indicators of the control group by 1.8 times ($p<0.01$). Tumor-α necrosis factor exceeded indicators of the control group by 2.2 times ($p<0.01$).

In the analysis of the relationship of blood lipid spectrum with markers of inflammation in patients with unstable angina was revealed the following correlative relationships: direct relationship of average power between STC and CRP ($r = 0.51$, p0.05); positive relationship of average strength between STC and IL-1β ($r=0,42$, $p<0,05$); direct relationship of average strength between STC and IL-6 ($r=0,44$, $p<0,05$); positive relationship of weak strength between STC and IL-17 ($r=0,16$, $p>0,05$); positive relationship of weak strength between STC and TNF-α ($r=0,19$, $p<0,05$); positive relationship of moderate strength between STC and fibrinogen ($r = 0.42$, $p<0.05$); direct relationship of moderate strength between LDLC and CRP ($r=0,40$, $p<0,05$); positive relationship of moderate strength between LDLC and IL-6 CRP ($r = 0.35$, p0.05); direct moderate relationship between LDLC and TNF-α ($r=0,36$, $p<0,05$); positive relationship of moderate strength between LDLC and IL-1β ($r = 0.33$, $p<0.05$); positive relationship of low strength between LDLC and fibrinogen ($r = 0.26$, $p<0.05$). The presence of correlational relationships between blood lipid spectrum and inflammation indicators points at their role and involvement in the pathogenesis of unstable angina, and may be a prognostically important factor in coronary heart disease.

DISCUSSION

The study of inflammatory markers is extremely topical for clinical practice, since they are necessary to confirm atherosclerosis or other related pathological conditions of the body: forecasting of the states, that precede serious complications (unstable angina, myocardial infarction), assessment of coronary atherosclerosis, its progression or regression. In our research we discovered a significant increase of proinflammatory cytokines: IL-1β, IL-6, IL-17, TNF-α and CRP, fibrinogen in the serum of patients with unstable angina to compare with control group. Our results reveal the activation of local and the development of systemic inflammatory process in patients with unstable angina.

Our results are comparative to the results of other authors. Therefore, Hashmi S. In his study observed an increase in proinflammatory cytokines (IL-17, IL-6, IL-8) and heatsensitive CRP and reduction of proinflammatory interleukin-10 of patients with unstable angina and acute myocardial infarction [22]. In another study, patients with coronary heart disease was noticed an increase in TNF-α, IL-6, and CRP to compare with a group of volunteers. In the same study, was explored the effect of excess TNF-α on monocytes in the blood and concluded the effect of TNF-α excess on monocytes in the blood was studied and there had been concluded that its excess is the main trigger mechanism to development of atherosclerosis and coronary heart disease [23]. In the experimental studies was shown that TNF-α, a proinflammatory cytokine, is involved in destabilizing the atherosclerotic plaque [24].

So, the research of inflammation in the pathogenesis of unstable angina, ischemic heart disease is important as it gives an understanding of the course of inflammatory processes in the body. It would be interesting to make study these indicators during the course of the disease; during treatment with medicines; the effect of drugs on the level of inflammation.

CONCLUSIONS

In patients with unstable angina was revealed a significant increase in serum levels of proinflammatory cytokines: interleukin-1β, interleukin-6, interleukin-17, TNF-α and C-reactive protein, fibrinogen, which reveals the activation of the inflammatory process. There was revealed a significant disturbance of blood lipid spectrum in patients with unstable angina. For its correction should be recommended diet and hypolipidemic medications.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

PECULIARITIES OF THE EFFECTS OF BILE ACIDS ON ATPASE ACTIVITY OF THE COLON MUCOSA IN PATIENTS WITH OVERWEIGHT AND IRRITABLE BOWEL SYNDROME

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ABSTRACT

The aim is to investigate the effect of bile acids on the ATPase activity of the colon mucosa in patients with overweight and irritable bowel syndrome (IBS).

Materials and methods: Completely examined 12 patients with IBS and overweight. We estimated the ATPase activity of colon mucous of the patients with IBS spectrophotometrically by determined the content of orthophosphate that was released after ATP hydrolysis. We studied the effect of 3-sulphate of taurolitocholate (TLC-S) on specific activities of Na^+/K^+-ATPase, Ca^{2+}-ATPase of endoplasmatic reticulum (EPR), Ca^{2+}-ATPase of plasmatic membrane (PM) and basal Mg^{2+}-ATPase of postmitochondrial subcellular fraction of colon mucous of the patients with IBS.

Results: We established the specific activities of Na^+/K^+-ATPase, Ca^{2+}-ATPase of EPR, Ca^{2+}-ATPase of PM and basal Mg^{2+}-ATPase. There were (6.06 ± 1.61), (5.88 ± 1.19), (8.86 ± 1.56) (6.44 ± 2.02) µmol P_i/ mg protein per hour, respectively. TLC-S (50 µM) did not caused any change of Na^+/K^+-ATPase , as well as Ca^{2+}-ATPases activities, but statistically significant increased activity of Mg^{2+}-ATPase of postmitochondrial subcellular fraction of colon mucous of the patients with IBS by 4 fold.

Conclusions: TLC-S increased basal Mg^{2+}-ATPase in the postmitochondrial fraction of colon mucous of the patients with overweight and IBS, but had no effect on Na^+/K^+-ATPase and Ca^{2+}-ATPases activities. It has been suggested that activation of basal Mg^{2+}-ATPase under by TLC-S may indicates the role of the endo-lysosomal system of epitheliocytes of colon mucous in developing of pathology IBS.

KEY WORDS: irritable bowel syndrome, overweight, ATPase, bile acids

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INTRODUCTION

Increased food intake and a reduction in energy expenditure are responsible for the increase in excess body weight and subsequent obesity. Today, according to the World Health Organization, over one billion people are overweight on the planet. In Ukraine, approximately one third of the population has excess body weight [1]. Obesity is the cause of various somatic diseases, in particular, the gastrointestinal tract, including gastroduodenitis with nausea and functional vomiting and irritable bowel syndrome (IBS), which is most often associated with restrictive eating behavior. According to various authors, the combination of obesity with dyskinesias of the colon with constipation, diverticular disease, colon polyposis was diagnosed, respectively, at 36,28; 28.0 and 10.0% of patients. Other researchers have found that in obese individuals an association with functional constipation occurred in 24.0% of cases, and obesity was observed in 60.0% of patients with constipation [2].

Obesity also develops against a backdrop of stress, serving as an indicator of psycho-emotional maladaptation and overcoming difficult life situations that are inhibited by excessive eating. In addition, excess body weight can act as a factor that prevents pleasure from life, and the latter phenomenon can be a factor that affects eating disorders, which in turn can contribute to the appearance of constipation, abdominal pain, changes in the sensitivity of serotonin receptors of the intestinal wall [2].

Obesity and a high body mass index have been shown to be significant risk factors for the development of IBS, in addition to insufficient amount of fiber in the diet, stress, inflammation, genetic predisposition [3]. Today, IBS is one of the most common diseases of the gastrointestinal tract, and obesity is an urgent problem of endocrinology [4]. IBS according to Rome Criteria IV is defined as a chronic functional bowel disorder characterized by recurrent abdominal pain, which occurs and continues at least once a week for the last three months, associated with bowel movements, changes in frequency and consistency of the stool [5].

An important factor in improving the diagnosis of IBS is to take into account the pathogenetic factors of the disease. In recent decades, perceptions of the pathogenesis of IBS have changed significantly. Previously, IBS was considered exclusively as a psychosomatic disease, and in almost all patients it was associated with the influence of psycho-emotional factors, but today the multifactorial development of IBS is obvious. Food allergies, stress, intes-

tinal infections, hereditary predisposition, malabsorption, and disorders of bile acid metabolism are the major triggers for the development of IBS [6]. Bile acids are amphipathic, detergent molecules synthesized by the liver that facilitate the absorption of lipids and fat soluble vitamins in the small intestine. Lithocholic and deoxycholic acids are the main bile acids present in the colon and feces. Henodeoxycholic and deoxycholic acids are known secretory bile acids. Increased excretion of feces and changes in the proportion of various bile acids in the feces characterize malabsorption of bile acids, which leads to diarrhea or IBS with diarrhea, which are associated with increased secretion of water and mucus in the colon, motility of the colon and membrane permeability. Bile malabsorption is known in 10–33% of patients with IBS with diarrhea or functional diarrhea [7].

However, the mechanisms of the link between metabolic regulation of bile acids and the pathogenesis of IBS remain unclear. Thus, studies that help identify specific pathogenetic mechanisms for the development of IBS are relevant.

THE AIM

The aim is to investigate the effect of bile acids on the ATPase activity of the colon mucosa in patients with overweight and irritable bowel syndrome.

MATERIALS AND METHODS

All procedures with patient were performed in accordance with the informed consent of the patient "International Convention for Working with Animals" under approval of the Bioethics Committee of DanyloHalytskyLviv National Medical University, protocol No2, 15/02, 2016.

Complex examination of 12 patients with IBS and excess body weight (mean age – 32,7 ± 1,5 years). The diagnosis of IBS was established according to Rome criteria IV [5] in the presence of recurrent abdominal pain, which was observed at least 1 day per week for the last 3 months and when there were two or more of the following symptoms: abdominal pain associated with bowel movements, pain accompanied by changing the frequency of stools or form of feces. For diagnosisof inflammatory bowel pathology, CITO TEST Calprotectin-Lactoferrin (Pharmasco) was performed. Wepayed attention to the absence of symptoms of anxiety: fever, impurities of blood in the stool, intestinal disorders, weight loss for a short period of time, anemia, leukocytosis, acceleration of erythrocyte sedimentation rate. All patients performed measurements of height and body weight. Body mass index was calculated by the Kattle formula. According to the obtained indicators, we established the presence of excess body weight.

Isolation of subcellular postmitochondrial fraction of the patients' colon mucous. Tissue samples were collected from patients colon during colonoscopy. Fresh samples were washed by medium A (mM): sucrose – 250, ethylene glycol tetraacetic acid (EGTA) – 1, HEPES – 10; KH_2PO_4 – 1; pH 7.2. Then these samples were homogenized with glass-glass homogenizer at 300 rev/min for 10 min at 0-2 °C. The homogenate was centrifuged for 10 min at 3.000 g using Jouan MR 1812 centrifuge (Jouan, France) to precipitate nuclei, large cells fragments, and undestroyed cells while mitochondria remained in the supernatant 1. Next centrifugation of this supernatant 1 carried out for 10 min at 8.500 g (0–2°C). After mitochondria sedimentation, supernatant 2 was used for while ATPase activity assay. To prove a membranes presence in the post-mitochondrial fraction it was sediment for 20 min at 15.000 g.

Assay of ATPase activity. ATPase activity was determined according to the content of orthophosphate that was released after ATP hydrolysis [8,9]. At the beginning of the experiment 200 μlof post-mitochondrial subcellular fraction of patients' colon mucous was transferred to a standard incubation medium containing (mM) NaCI – 50.0; KCI – 100.0; Tris-HCI – 20.0; $MgCI_2$ – 3.0; $CaCI_2$ – 0.01; pH 7.4 at 37 °C. The reaction was started by adding3 mMATP(Sigma, USA). Samples were incubated for 15 minat 37 °C withmoderateshakingin a water bath. Before theend ofincubation0.4ml of mediumwas takenfor the determination ofprotein content by Lowry [10]. Reactionwas stopped byadding 5ml of 10% trichloroaceticacid to samples and incubating themfor 30 minfollowed by 10 min centrifugationat 1600 g. Supernatant obtainedwas usedto determine thecontentofinorganic phosphorusby the spectrophotometric method ofFiske-Subbarow[11]. We used TLC-S(Sigma, USA) at concentration 50 μmol/L for estimating their effect on ATPase activity.

Calculation of ATPase activity. The total ATPase activity of post-mitochondrial fraction of colon mucous was calculated by the difference of inorganic phosphorus in the medias with different composition (supplemented with TLC-S – "experiment" or not supplemented – "control") expressed as micromoles of inorganic phosphorus equivalent to 1 mg of protein per 1 h. Specific Na^+/K^+-ATPase activity was calculated as difference of inorganic phosphorus content in medium with or without ouabain (1 mM). For thedetermination ofCa^{2+}/Mg^{2+}-ATPase activity,the differencebetween the totalCa^{2+}/Mg^{2+}-andNa^+/K^+-ATPaseactivity was quantified.Thapsigargin was used to calculate SERCA contribution into the total Ca^{2+}/Mg^{2+}-ATPase activity. Specific basal Mg^{2+}-ATPaseactivitywas determinedin incubationmediumthatcontained1 mMEGTA and lackedouabain. In allexperiments,incubation mediumwas as a controlforthe enzymaticATP hydrolysis.

Data analysis. The significance of differences between experimental groups was calculated using Wilcoxon-Mann-Whitney, when a data distributions were not normal. P≤0.05 was considered to be statistically significant.

RESULTS

It was found that *Na^+/K^+-ATPase* activity of subcellular fraction of colon mucous ranged from 2.32 to 15.76 and averaged (6.06 ± 1.61) μmol Pi/ mg protein per hour. TLC-S caused ranging of *Na^+/K^+-ATPase* activity from 0.74 to 13.99 and averaged (7.62 ± 1.64) μmol Pi/ mg protein per hour. Therefore, no statistically significant changes were found by

bile acid on the activity of *Na^+/K^+-ATPase* of the subcellular fraction of the colon mucous of patients with IBS.

We observed that the Ca^{2+}-ATPase activity of EPR was ranging from 0.28 to 14.14. It was equal in average (5.88 ± 1.19) μmol Pi/ mg protein per hour. TLC-S adding to the incubation medium resulted in fluctuations its activity from 0.23 to 10.89 and averaged (6.51 ± 1.20) μmol Pi/ mg protein per hour. It was found that Ca^{2+}-ATPase activity of PM in control ranged from 4.84 to 15.34 and averaged (8.86 ± 1.56) μmol Pi/ mg protein per hour. When TLC-S was added to the incubation medium, the activity rates of this pump ranged from 0.61 to 10.49 and averaged (6.16 ± 1.34) μmol Pi/ mg protein per hour.

We found that basal Mg^{2+}-ATPase activity in postmitochondrial subcellular fractions of colon mucous of the patients with IBS ranged from 0.42 to 9.24, which averaged (6.44 ± 2.02) μmol Pi/ mg protein per hour. Addition of TLC-S to the incubation medium resulted in fluctuations in the activity of basal Mg^{2+}-ATPase activity in the range from 5.16 to 32.6 and averaged (23.19 ± 5.22) μmol Pi/ mg protein per hour.

DISCUSSION

Influence of TLC-S on Na^+/K^+-ATPase activity in postmitochondrial subcellular fraction of colon mucous of the patients with IBS. As *Na^+/K^+-ATPase* plays an important role in electrolyte, water and nutrient transport across the intestinal epithelia, it is expected that the any changes in *Na^+/K^+-ATPase* activity may have a major impact in intestinal function, namely absorption and secretion. It was shown that activities of *Na^+/K^+-ATPase* was increased in children with toddler diarrhea, but *Na^+/K^+-ATPase* activity was reduced in the jejuna mucosa of patients with active celiac disease [12]. So the role of activities of *Na^+/K^+-ATPase*in IBS pathology still unknown. It is consider that perturbed *bile acid* metabolism plays a causal role in *IBS* [13]. It is possible to suppose that TLC-S might effect on activity of Na^+/K^+-ATPase in postmitochondrial subcellular fraction of colon mucous of the patients with IBS. But we did not found the effect of TLC-S on the activity of *Na^+/K^+-ATPase* of the subcellular fraction of the mucous membrane of the colon in patients with IBS. Our results are agreed with Hafkenscheid, who found that "the taurine derivates TC, TCDC and TDC did not influence or even enhanced the *Na^+/K^+-ATPase* activity " [14].

Influence of TLC-S on total Ca^{2+}-ATPases activity in postmitochondrial subcellularfraction of colon mucous of the patients with IBS. The extracellular Ca^{2+} influx is balanced by Ca^{2+}released from the cytosol by both plasma membranes and the internal Ca^{2+} -ATPases. The total Ca^{2+} -ATPases activity of the subcellular fraction consists of EPR Ca^{2+} -ATPase and plasma membrane (PM) Ca^{2+} pump EPR Ca^{2+} -ATPase play an essential role in the transport of $Ca^{2+}$to the EPR to replenish the calcium store, promote folding and protein maturation, lipid and steroid synthesis. It is known that TLC, as well as TLC-S, mobilizes Ca^{2+} from the intracellular pool. Thus, the main effect of TLC-S is associated with an increase in calcium cells and depletion of calcium stores. Therefore, TLC-S should affect the activity of Ca^{2+} -ATPases of the subcellular fraction of colon mucous too. But we did not observe the influence of TLC-S on Ca^{2+}-ATPase activity of the subcellular fraction of the colon mucous membrane of patients with IBS.

Influence of TLC-S on basal Mg^{2+}-ATPase activity in postmitochondrial subcellular fractions of colon mucous of the patients with IBS. It should to note that activity of basal Mg^{2+}-ATPase activity is coupled to H^+-translocation in PM [15,16] as well as in endosomal fraction [17]. Also in hepatocytes Mg^{2+}-ATPase activity is considered as markers of canalicularmemebrane [18]. Mg^{2+}-activated ATPase of rat colon was studied in mucosa by J.Schreiner and coautors & Hafkenscheidin [14, 18] and in muscle loyer by Kaplia 2017 [19]. It was shown that all bile acids except cholic acid, taurocholic acid and chenodeoxycholic acid depressed the Mg^{2+}-ATPase activity in rat colon mucosa [14].

We found a statistically significant increasing of the activity of basal Mg^{2+}-ATPase activity in subcellular fraction of colon mucous under the action of TLC-S compared with the control by 3.6 times. The obtained results by the effects of TLC-S are in full agreement with the previously observed the effect of TLC-S on the activity of basal Mg^{2+}-ATPase activity in the subcellular fraction of rat liver [20].

It has been suggested that activation of basal Mg^{2+}-ATPase under the action of TLC-S may indicates to the role of the endo-lysosomal system, the so-called acid store of colon mucous of the patients in developing of pathology IBS.

CONCLUSIONS

TLC-S (50 μM) increased basal Mg^{2+}-ATPase in the postmitochondrial fraction of colon mucous of the patients with overweight and IBS, but had no effect on Na+/K+-ATPase and total Ca^{2+}-ATPases activity.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

EFFICACY ASSESSMENT OF THE SCHEME FOR PREVENTION OF HERPESVIRUS INFECTION MANIFESTATIONS IN THE ORAL CAVITY OF PATIENTS WITH HERPES-ASSOCIATED GENERALIZED MODERATE SEVERITY PERIODONTITIS

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ABSTRACT

The aim: Efficacy assessment of herpesvirus infection drug prophylaxis (HVI) manifestations in the mouth of patients with chronic herpes-associated generalized periodontitis (CHGP) of moderate severity during and shortly after closed curettage.

Materials and methods: The total of 87 patients with CHGP of moderate severity aged 35-60 years were examined and divided into groups according to the presence of HVI: Group I (main) included 48 patients who had herpesvirus infection; group II (comparison) – 39 patients who were not found herpesvirus infection. Group III was formed based on the data of out-patient medical reports retrospective analysis and was used to compare the number of complication cases. The control group included 20 patients with intact periodontium.

Prior to treatment, patients in group I, was additionally assigned multicomponent phytocomplex. The treatment efficacy was assessed by the concentration dynamics of A, G, M immunoglobulins, circulating immune complexes (CIC) and sIgA, hygienic and periodontal indices, as well as by the dynamics of gum fluid and the recovery terms.

Results: The study on the dynamics of clinical indices and some humoral immunity indices confirmed the main group patients after closed curettage had positive shifts in sIgA, IgA, IgG, IgM and CIC levels. Their indices did not have a statistically significant difference with similar indicators of the comparison group. In group I, complications in the form of HVI manifestations in the buccal mucous membrane (BMM) were found in 8.33% of patients, which had a statistically significant difference ($p < 0.001$) from the percentage of patients with HVI, with complications in group III (35.71%). The recovery terms for patients in group I were by 16.7% shorter than the similar terms in group III.

Conclusions: The results obtained indicate that the phytocomplex used by us can be applied as an immunomodulatory agent for the prevention of herpes virus infection manifestations in the oral cavity of patients with interventions in BMM and periodontal tissues.

KEY WORDS: generalized periodontitis, curettage, herpes virus infection

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INTRODUCTION

According to the WHO, 70 to 90% of the world's population is infected with one or more herpes virus types and 50% of them have a recurrence of the disease every year due to the lack of persistent immunity.

According to Slots et al. (2002) representatives of *Herpesviridae* viruses (1 and 2 types herpes and cytomegalovirus) constitute the microflora of the periodontal pocket in patients over 45 years of age with chronic generalized periodontitis. In 80% of patients with chronic generalized periodontitis, signs of secondary immune deficiency are observed in the exacerbation phase against the background of recurrent herpes virus infection.

A number of researchers have confirmed the role of immune mechanisms in the pathogenesis of inflammatory periodontal diseases caused by viral-bacterial association [1, 2, 3]. The interaction between herpes virus and bacterial flora is likely to have a bidirectional correlation, taking into account the activity of bacterial enzymes and other factors that induce inflammation and cause involvement of herpes virus in the pathology development. Herpes viruses can cause periodontal tissue disease directly – both as a result of the virus infection and its replication in the body, and as a result of the indirect impact of virions on the body's protection system.

Patients affected by chronic herpes virus infection not only have a more severe periodontal disease course but also a deeper nature of the immune system changes compared to patients who have similar periodontal tissue pathology but are not HVI-carriers, as evidenced by the analysis of numerical studies on the cell and humoral components of the immune system [4,5].

For the comprehensive treatment of herpes virus infection, nucleoside analogues are used, as a rule, as antiviral chemotherapy drugs. They block the herpesviruses replication by suppression of viral DNA polymerase and inhibiting DNA synthesis. However, these drugs only act on the virus at the stage of active replication, have low bioavailability and a number of toxic effects. Today, an increasing number of viral strains resistant to the action of these drugs are recorded [6, 7, 8].

The reason to refuse drugs for external use only in treatment of herpes virus infection was the increasing incidence of herpes infection overt symptoms exacerbations, which is now widely recognized and documented in international recommendations. The efficacy of treating chronic generalized periodontitis associated with persistent herpes virus infection depends not only on the specific antiviral drugs used in the case of herpes virus infection in the virus carriers' oral cavity, but also on the immunocorrection therapy. Therefore, the problem of developing and applying the existing prevention regimens for these conditions remains relevant.

THE AIM

The purpose of this study was to assess the efficacy of the scheme developed by the authors for the drug prevention of herpes virus infection manifestations in the oral cavity of patients with chronic generalized periodontitis of moderate severity during surgical treatment (closed curettage) and in the short term after it.

MATERIALS AND METHODS

Materials and methods of the study. The study is carried out in compliance with the main provisions of the GCP ICH and the Helsinki Declaration on Ethical Principles for Medical Research Involving Human Subjects, and its subsequent revisions (Seoul, 2008), the Council of Europe Convention on Human Rights and Biomedicine (2007) and the recommendations of the Committee on Bioethics at Presidium of the NAMS of Ukraine (2002) and the positive opinion of the Shupyk NMAPE Committee on Ethics (excerpt from Minutes No. 2 of 10/01/2017).

To fulfil these tasks, 87 patients with chronic generalized periodontitis (CGP) of moderate severity aged 35 to 60 years were examined.

Within the framework of the study, for the purpose of comparative analysis, we also performed a retrospective analysis of 28 out-patient medical records of patients who were herpes virus carriers and were treated for moderate-severity CGP with the use of closed curettage by the traditional method [9].

The control group consisted of 20 patients who had no periodontal tissue disease and consulted a dentist for treatment of uncomplicated caries.

Verification of the "generalized periodontitis" diagnosis was carried out based on conventional clinical and paraclinical examination methods with determining of periodontal PI index III (by Russel), PMA (by Parma), gum recession assessment (by P.D. Miller). Oral hygiene status was assessed using the Green – Vermillion index. The diagnosis was confirmed by radiography.

Patients whose depth of periodontal pockets did not exceed 4 mm were involved into the study. Before treatment, patients were asked to complete a questionnaire, which included questions about the presence of somatic diseases, bad habits (smoking), and the presence or absence of herpes virus manifestations in the patient during the last ten years of life.

According to the previous history it was established that 48 patients had manifested symptoms of herpes virus infection during their lifetime. Verification of the persistent herpes virus infection (HVI) diagnosis was performed based on anamnesis assessment, clinical course of the disease, differential diagnosis with similar diseases, enzyme immunoassay (EIA) and polymerase chain reaction (PCR).

Depending on the presence or absence of herpesvirus infection, patients were divided into two groups: Group I (main) included 48 patients who had herpesvirus infection; group II (comparison) – 39 patients who were not found herpesvirus infection. Group III was formed based on the data of out-patient medical reports retrospective analysis and was used to compare the number of complication cases in the form of herpes virus manifestations in the oral cavity during the closed curettage and the recovery terms after the use of traditional therapy methods without immunomodulating and antiviral drugs. The homogeneity of the patient groups was assessed in terms of their comparability on demographic and clinical grounds and was determined for the correctness of the treatment efficacy comparative analysis.

The immune protection status of patients in the both groups was determined by studying the concentration of A, G, M immunoglobulins, circulating immune complexes (CIC) in peripheral blood and sIgA in the oral fluid. The treatment efficacy was assessed by the dynamics of hygienic and periodontal indices, as well as by the gum fluid dynamics indices and by the recovery terms.

RESULTS

Objective examination of the oral cavity in groups I and II patients revealed the presence of soft dental deposits and mineralized supra- and subgingival dental deposits. The hygiene index in the main group was worse (22.1%) than that in the comparison group and had a statistically significant difference with the similar index in the control group. The clinical picture was characterized by gums hyperemia with pronounced cyanosis, pronounced edema, disturbed turgor of gum tissue and the gingival margin relief, gum pain and bleeding, traumatic occlusion. More intense gum inflammation and more pronounced clinical tissue changes of the periodontal complex were determined in group I patients.

The periodontal indices values in the patients belonging to this group significantly exceeded the values of similar indices in the comparison group patients: PMA – by 28.29% ($p < 0.001$); RVI – by 12.14% ($p < 0.001$); RI – by 23.9% ($p < 0.001$). The mean depth of periodontal pockets in patients of group I was 3.72±0.34 mm, whereas in patients of group II they were 3.28±0.31 mm. The amount of gum fluid in the gingival crevice and in the periodontal pocket of group I patients also exceeded the similar index in group II by 1.57 times ($p < 0.001$), which indicated a greater severity

of the inflammatory process in the periodontal margin of individuals with CGP associated with persistent herpes virus infection.

The study found that A (IgA) immunoglobulin values in group I patients exceeded those in the control patients by 1.35 times and in group II patients – by 1.06 times ($p < 0.05$). G (IgG) immunoglobulin values also exceeded those in the control group by 2.08 and in group II – by 1.7 times ($p < 0.001$), respectively. M (IgM) immunoglobulin scores were also by 2.23 times higher than in the control group and by 1.7 times higher ($p < 0.001$) than in group II. The values of circulating immune complexes (CIC) in group I patients also exceeded the similar indices in the control group patients by 4.14 and by 3.28 times ($p < 0.001$) in group II, respectively.

Values of sIgA indices in the oral fluid also differed in group I (0.081±0.006) and group II (0.097±0.007) and had a statistically significant difference with those of the control group (0.174±0.02) ($p < 0.001$).

The above studies suggest that an increase in IgA, IgG, IgM levels and a decrease in sIgA levels in patients of groups I and II are due to the presence of the bacterial infection as a pathogenic link in the development of inflammatory periodontal tissue diseases. In addition, in group I (main), this index was also influenced by persistent herpes virus infection, which also causes an increase in the serum immunoglobulin level and a decrease in sIgA titer in the oral fluid. The high CIC concentration in group I patients is explained by the fact that prolonged persistence of bacterial and viral infection in the body is accompanied by the immune system activation with formation of CIC, which subsequently re-trigger activation of cellular and humoral components of the immune system and, as a consequence, autoimmune response development.

All patients in phase I of generalized periodontitis treatment received standard local therapy, which included removal of supra- and sub-gingival calculus, correction of butts and elimination of other traumatic factors. After the inflammatory process subsidance, carious cavities were sanitated, traumatic occlusion items were removed [10 11]. Drug treatment included oral irrigation with antiseptic solutions, antibacterial therapy for periodontal pockets, anti-inflammatory and reparative treatment.

Prior to treatment, patients in group I, who were diagnosed with herpes virus infection, was additionally assigned multicomponent phytocomplex "Dzherelo-I" containing water-alcohol standardized extracts of aloe, plantain, sage, nettle, knotgrass, yarrow, echinacea, hypericum, organy, absinthium , sandy everlasting, thyme, bur beggar-ticks, calendula flowers, berries of viburnum, buckthorn, fruits of sweet-brier, fennel, juniper, roots of blowball, rhodiola, licorice, sweet calamus rhizome, rhizomes and roots of elecampane, tormentil rootstock, chaga (phytoextract). The purpose of the phytoextract prescription was to prevent the onset of herpes virus pathology manifestations in the oral cavity of patients at the stages of moderate severity CGP treatment. The drug has antimicrobial, antiviral, anti-inflammatory effect, significantly enhances the reparative function of immunity. Moreover the more pronounced immunosuppression is the stronger is the potentiating effect of the drug. The drug has good compatibility, it can be used not only on its own, but also with other drugs in the comprehensive treatment of most diseases. When applied to the skin and mucous membranes, the drug quickly reduces inflammation. The drug was prescribed for a comprehensive course of 21 days: for internal administration – 50-70 drops per 100 ml of water 2 times a day 40 minutes before meals; and for rinsing the oral cavity – in the ratio of 20-30 drops per 1 tablespoon of water. To consolidate the treatment effect, patients underwent closed curettage of periodontal pockets. At the time of surgery, patients in both I and II groups had an equivalent clinical picture of the disease course.

In order to prevent the development of systemic septicemia and to avoid bacterial endocarditis, patients were prescribed prophylactic oral administration of clindamycin: 600 mg for 60 minutes [11]. The operation of closed curettage for both groups was performed in the area of four incisors in the mandible according to the conventional method and using local anesthesia with a solution of articaine hydrochloride and irrigation with a warm solution of antiseptic (0.05% solution of chlorhexidine bigluconate).

Patients of group I (main) and group II (comparison) received postoperative drug therapy according to the schemes, suggested and developed by us, which included oral administration of the serratiopeptidase drug.

The treatment regimen included: washing the periodontal pocket with a warm antiseptic solution (0.05% solution of chlorhexidine bigluconate); instillation of the dental treatment paste into periodontal pockets in the surgery intervention site (mefenamic acid, vinylin – 2.0; zinc oxide – 2.0), which was prepared immediately before use; applying adhesive bandage (Reso-pac); assignment of serratiopeptidase for oral administration at the dose of 10 mg three times a day 40 minutes before meals for 8 days. Patients of group I were additionally assigned "Dzherelo-I" phytocomplex solution to rinse mouth and for gingival applications in the proportion of 20 – 30 drops for each tablespoon of warm water (solution prepared immediately before use and not stored) three times a day for three weeks. The control examination of patients was carried out on the second day after the surgery. The term of the second stage of surgery depends on the complaints and the clinical picture in the operation site.

At the second stage of surgery, the proposed regimens were supplemented with vitamin preparations (retinol acetate 3.44%, tocopherol acetate 10%) and reparative drugs (dental gel containing deproteinized hemoderivate of the calves blood). Patients of group I were additionally prescribed applications of the "Dzherelo-I" phytocomplex solution for 30 minutes 2 times a day to the diseased site. Patients were given recommendations on the features of nutrition and oral hygiene.

After the first stage of closed curettage, accompanied by the appropriate medical treatment, in group I, complications in the form of herpesvirus infection manifestations

on the oral cavity mucous membrane, inflammatory and exudative phenomena were revealed in 4 patients (8.33%) with moderate and severe herpesvirus infection course, which had a statistically significant difference (p <0.001) with the percentage of HVI-patients with acute complications in group III (10 patients amounting 35.71%).

In addition, these patients complained of pain in the intervention site. For these patients, the second stage of closed curettage was delayed until the lesions epithelialization and was performed 6±2.18 days after the first stage. To treat manifestations of herpes virus infection, this patient was prescribed inosine pranobex 500 mg at the dose of 2 tablets 4 times a day for 14 days. No complications were found in patients of group II.

Patients of groups I and II who had no complications after the first stage of closed curettage, were performed the next stage three days after.

After the second stage of closed curettage, inflammatory exudation and pain in the intervention site disappeared on the 2nd-3rd day. As a rule, on the third day the signs of a pronounced inflammatory process subsided. Postoperative wound healing occurred within 5±1.26 days after the manipulation in patients of group I and after 4.3±1.16 days in patients of group II, as evidenced by the normalization of clinical indices presented in table 1. When comparing the recovery terms in patients of groups I and III a statistically significant difference was found in the recovery terms. It was 5±1.26 days in Group I versus 6±2.18 days in Group III.

The assessment of the nearest clinical results in treatment of moderate severity CGP according to the suggested regimens in patients of groups I and II was carried out within 7 days after the second stage of closed curettage. Along with clinical indices, changes in the immune protection status in both groups were assessed. The values of sIgA, IgA, IgG, IgM and CIC levels in both groups I and II also had positive dynamics. Thus, as a result of treatment performed in group I, the level of sIgA increased significantly by 39.55%.

The level of IgA decreased by 12.23%; IgG – by 40.52%; IgM – by 42.14%. In group II, on the 7th day after the second stage of closed curettage, the levels of immunoglobulins decreased: IgA – by 16.93%; IgG – by 40.29%; IgM – by 18.58%. The level of sIgA increased by 38.22%. The CIC level indices also had positive dynamics in both group I and group II. In group I, the reduction of the CIC level was 61.15%; in group II – 59.71%. Dynamics of the humoral immunity indices of patients in groups I and II

Table I. Dynamics of clinical indices in patients of groups I and II before and in the nearest terms after treatment

Indices	Control group (n=20)	Group I (n=48)		Group II (n=39)	
		Before treatment	After treatment	Before treatment	After treatment
IG	0.32±0.08	3.44±0.44	0.93±0.04***	2.68±0.09	0.36±0.08***
PI	0.1±0.002	4.39±0.14	1.67±0.06***	3.34±0.19	0.54±0.07***
PBI	-	3.21±0.06	1.48±0.18***	2.82±0.11	1.01±0.21***
PMA	6.31±0.61	44.89±1.18	18.10±2.18***	32.19±1.54	7.12±1.64***
Amount of crevicular fluids	0.42±0.02	2.42±0.14*	1.3±0.05**	2.1±0.086	0.9±0.05**

Note. Reliability of the difference between the indices of the main, the comparison and the control groups: * - p <0.05;** - p <0.01 ***- p <0.001

Table II. Dynamics of humoral immunity indices in patients of groups I and II before and in the nearest terms after treatment

	Immunological indices				
	sIgA	Ig A	Ig G	Ig M	CIC (units)
Healthy periodontium (n=20)	0.174±0.02	1.48±0.32	13.95±2.19	1.71±0.14	34.1±4.19
			I **main group (n=48)**		
Before treatment	0.081±0.006	2.01±0.31	29.12.±1.59	3.82± 0.16	141.1±17.1
On the 7th day after stage II	0.134±0.03 P1<0.01 P2<0.05	1.76±0.35 P1<0.01 P2<0.05	17.32±0.94 P1<0.01 P3<0.01	2.21±0.18 P1<0.01 P3<0.05	54.81±4.27 P1<0.001 P3<0.05
			II **comparison group (n=39)**		
Before treatment	0.097± 0.007	1.89±0.26	23.8±1.28	2.26±0.31	102.04±12.36
On the 7th day after stage II	0.157±0.03 P3<0.05	1.57±0.21 P3<0.01	14.21±0.77 P3<0.01	1.84±0.25 P3<0.05	41.11±2.61 P3<0.01

Note. P1- reliability of difference from the control group (healthy); P2- reliability of difference between the main and the comparison groups; P3- reliability of difference within the group between the indices before and after treatment.

before and in the short term after the treatment are presented in table 2.

DISCUSSION

In general, our study results are consistent with the data of other authors who studied manifestations of herpes virus infection in the oral cavity (Volosovets T.M., 2013, Petrushanko T.O, Skrypnikov P.M, Litovchenko I.Yu. et al., 2014) in the periodontal tissues pathology [5, 12]. The efficacy of the "Dzherelo-I" phytocomplex has been proven by numerous studies performed at the Luhansk Regional Center for AIDS Prevention and Control (Chkhetiani R.D. 2003, 2004), Institute of Neurosurgery and Institute of Pediatrics, Obstetrics and Gynecology, NAMS of Ukraine, (Lisyanyi M.I., Pylypchuk V.S., Belska L.M. et al. 2018). It was designed as an adjunct to prevent the onset of herpesvirus pathology manifestations in the oral cavity of patients at treatment of moderate severity CGP. The data obtained during our studies are consistent with the results of the above authors [13].

CONCLUSION

The study on the dynamics of clinical indices and some humoral immunity indices confirmed that patients of group 1 (main) had positive shifts in the concentration of sIgA in the oral fluid and IgA, IgG, IgG in the peripheral blood on the 7th day after the second stage of closed curettage. In terms of their values, there was no statistically significant difference with similar patients in the comparison group. When comparing the number of complications and the recovery terms in patients of group I to the similar indices of group III patients, it was found that in group I complications in the form of manifestations in the oral mucosa were observed in 8.33% of persons with moderate and severe HVI course, which had a statistically significant difference ($p < 0.001$) from the percentage of patients with acute complications in group III (35.71%).

Thus, the results obtained by us indicate that the "Dzherelo-I" phytocomplex, which contains a standardized number of phyto-drugs, has a pronounced antiviral, anti-inflammatory effect, significantly enhances the reparative function of the oral mucosa. In the comprehensive treatment of moderate severityCGP, it can be used as an immunomodulatory agent for prevention of herpes virus infection manifestations in the oral cavity of patients with interventions in the oral mucosa and periodontal tissues.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

USE OF TECHNOMOLECULAR SILVER PREPARATIONS IN COMPLEX TREATMENT OF INFECTED WOUNDS

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ABSTRACT

The aim: To improve the results of treatment of infected wounds.

Materials and methods: The clinical material is based on clinical observation and treatment of 29 patients with in fected wounds, whose treatment included combination drugs of local action on the basis of techno-molecular silver (in particular «Cadefort-Spray»), by application to the wound surface.

Results: Wound microbial factor, dynamics of wound process, indicators of immune status were evaluated: localadaptive immunity, atopic reactions.

Conclusions: High efficiency of treatment was observed regardless of the phase of the wound process, which allowed to accelerate wound repair and stimulate the processes of regeneration, strengthen local adaptive immunity, prevent atopic reactions.

KEY WORDS: wound, silver ions, regeneration, local immunity, atopic reactions

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INTRODUCTION

In recent years, the problem of treating infected wound injuries has augmented significantly, both in the world and in Ukraine in particular. Such an amplification of the problem is evidenced by the increase in the number of surgical patients with purulent-inflammatory diseases by up to 35-40%, and the total lethality from purulent infection by up to 42-60% [1,2]. The proportion of surgical wound infection accounts for up to 15% of the total number of nosocomial infections [1,2,3].This is primarily due to the increase in the number of patients with vascular disorders (atherosclerosis, chronic venous insufficiency), to the rapid development of antibiotic-resistant microflora and to a significant decrease in human immune protection, since the mutagenic effect of antibiotics on pathogenic microflora has led to a change in the etiological structure of purulent surgical infection and biological properties of the microbial cell, and environmental contamination has led to the immunobiological resistance of the macroorganism [1,2,4]. Modification of virulence and resistance of microorganisms require improvement of method sandways of surgical treatment of purulent-necrotic processes against the background of the burdened general condition of the patient. The severity of purulent-necrotics of ttissue disease in most patients is due to the additional impact of major background pathology, such as chronic intoxication, decompensation of comorbid somatic diseases, poly-organ dysfunction syndrome, and secondary immunodeficiency [4,5].

In this regard, drugs of local action of the antibiotic spectrum have been surely losing their strong positions until recently [4,5]. In such conditions, the situation arises when the possibilities of correction of metabolic disorders and treatment of the underlying disease, as well as carrying out systemic etiotropic antibacterial therapy, are significantly limited [5,6] due to the deterioration of the detoxification potential, the compensatory and adaptive properties of the organism. Therefore, the need to improve the effectiveness of local treatment of the cell of destruction and purulent inflammation is increasing. In this regard, the agents of local action, the antibiotic spectrum, are definitely losing their until recently strong positions. The current level of theoretical and practical advances in surgery allows us to look from new perspectives on the problem of improving the effectiveness of therapeutic agents intended for the treatment of wounds. This allows a differentiated approach to the choice of treatment methods, taking into account the pathogenesis and phases of the wound process, as well as the nature of the pathogen and its sensitivity to drugs. [5,6].

THE AIM

The goal of this study was to analyze the features of pathogenesis and the course of the wound process of modern infected wounds; to improve the results of treatment of infected wounds by the use of preparations based on techno-molecular silver, with the purpose of effective suppression of microflora, limitation of inflammation, purification from purulent-necrotic content in the short term taking into account the phases of the wound process. Another goal was to improve local adaptive immunity and prevent the development of atopic reactions.

MATERIALS AND METHODS

The clinical material is based on the clinical observation and treatment of 39 patients with infected wounds of different origins at the Clinic of the Department of General Surgery and Surgical Department of the Clinical Hospital "Uzhhorod Regional Hospital" for 2018-2019.

In 17 patients, chronic vascular disorders of the lower extremities were the cause of the wound injury. In 14 patients the cause were purulent-necrotic lesions of subcutaneous fat such as carbuncles, boils, abscesses. In 8 patients, burn wounds were due to thermal damage. The control group consisted of 10 patients with infected wounds, whose treatment was carried out by the classical method, without the use of silver preparations. For the surgical treatment of wounds and trophic ulcers, antiseptic solutions (0.02% solution of decasan, 0.05% solution of rivanol, 0.5% aqueous solution of chlorhexidine bigluconate locally) and various powder application sorbents were used in the exudation phase.

RESULTS AND DISCUSSION

Bacteriological examination of the content of wounds revealed a predominance of pathogenic Staphylococcus aureus and its associations, in particular with Proteus, Escherichia coli or Pseudomonas aeruginosa (Fig.1).

Inthecontrol group, the granulation tissue developed an average of 6.5 ± 0.42 days. Of these, ten (29.4%) revealed juicy granulations, sixteen (47.0%) showed brittle (a small number of vessels, a lot of amorphous substance and cellular elements), and juicy granulations (a considerable number of newly formed ones). Vessels with a small number of cellular elements) in these patients appeared on the average only on 8,2 ± 0,29 days. Eight patients (23.6%) had no granulation at all within 12 days, and appeared with an average on 16.59 ± 0.55 days. The wounds were cleaned by an average of 5.15 ± 0.35 days, their size decreased daily (measurements were performed with a sterile centimeter tape) by an averageof 1.1 ± 0.07 cm.

In the treatment of the main group (19 patients), along with the background use of conventional therapeutic measures of local and general influence, combination drugs of local action, on the basis of technomolecular silver (in particular "Kadefort-Spray"), were applied to the woundsurface once a day. Due to the presence of silver, kaolin, sodium hyaluronate and chlorhexidine ions in its composition, Kadefort-Spray allows to stimulate and accelerate regenerative processes against the background of stable antiseptic and dehydration effect (Fig. 2, 3, 4).

Control of therapeutic dynamics was based on the course of the wound process as compared toaverage wound healing data, which was halved. We observed faster appearance of mature granulation tissue and of signs of marginal and islet epithelialization.

It was noted that due to its content of hyaluronic acid, kaolin and technomolecular silver, "Cadefort-spray" has a positive effect on the wound process irrespective of phase and destroys microorganisms at the cytological level. According to the bacteriological study, the disappearance of pathogenic microorganisms in the wound was observed at 7-10 days of the healing process. Due to the film-forming, Cadefort Spray protects the wound surface from further daily mechanical damage and has an indirect analgesic effect, which is especially important in the treatment of burn wounds (Fig. 5, 6).

The level of body's defenses was assessed by indicators of the immune status of the organism, which was the evidence base for the study. The number of T-helpers (CD-4), T-suppressors (CD-8), their ratio (Tx (CD-4) / Tc (CD-8), as an indicator of local adaptive immunity, and IgE dynamics as a marker were taken into account. The analysis of immunological data showed that patients with chronic skin damage demonstrated damage of their immune status.

In these patients, against the background of a decrease in the total number of lymphocytes, the following were noted: a sharp decrease in the amount of Tx (CD-4) with a moderate decrease in Tc (CD-8), a corresponding decrease in their ratio, and a decrease in the level of Ig E.

After treatment, there is a tendency of normalization of the CD-4 / CD-8 ratio (Fig.7) and an increase in IgE levels (Fig.8), which indicates an increase in local adaptive immunity and absence of atopic reactions.

CONCLUSION

Intoday's treatment of patients with infected wounds, it is necessary to take into account the rapid development of antibiotic-resistant microorganisms and the global weakening of the immune protection of the population. The choice of modern topical applications makes it possible to significantly improve the results of treatment, with minimal financial costs and minimal toxic effects on the patient's body, to reduce the patient's stay in hospital, to increase local adaptive immunity, and to prevent the development of atopic reactions.

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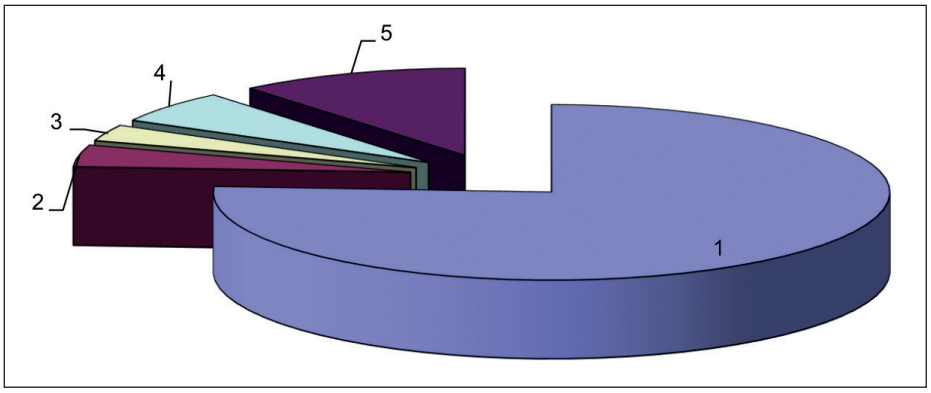


Fig. 1. The nature of the microflora in wounds: 1 – Staphylococci (76%); 2 – Pseudomonas aeruginosa (6%); 3 – Proteus (3%); 4 – Escherichia coli (4%); 5 – microbial associations (11%).

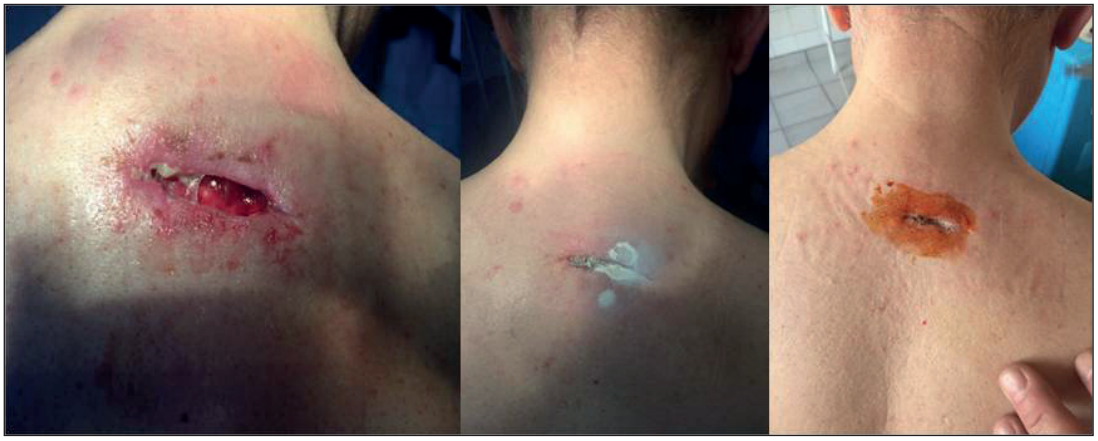


Fig. 2. An infected wound. Early dynamics. The treatment period is 11 days.

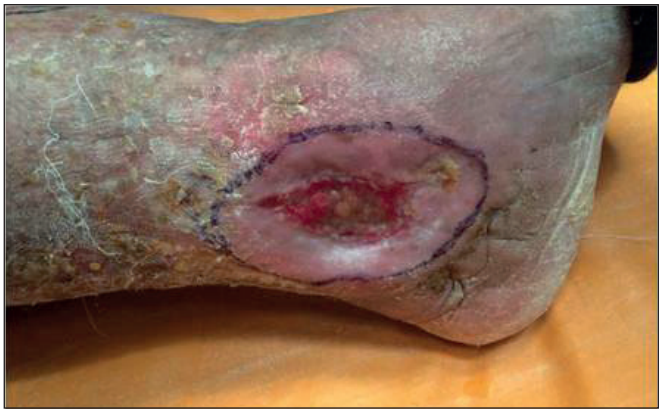


Fig. 3. Trophic ulcer of the shin. 14 days of treatment. Signs of regional epithelialization.

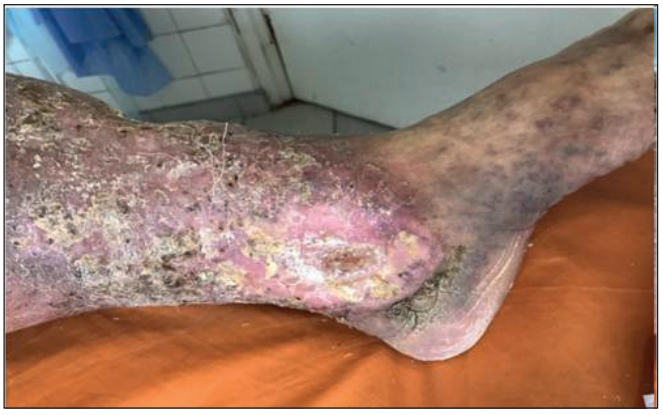


Fig. 4. Trophic ulcer of the shin. 19 days of treatment.

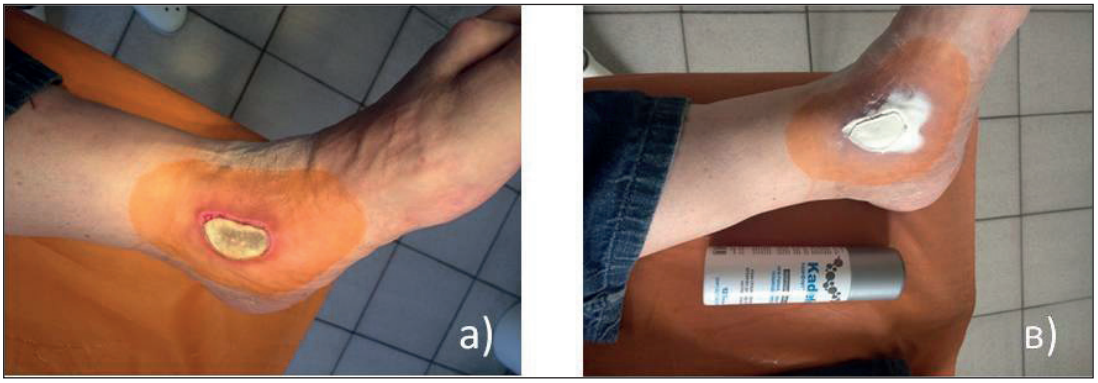
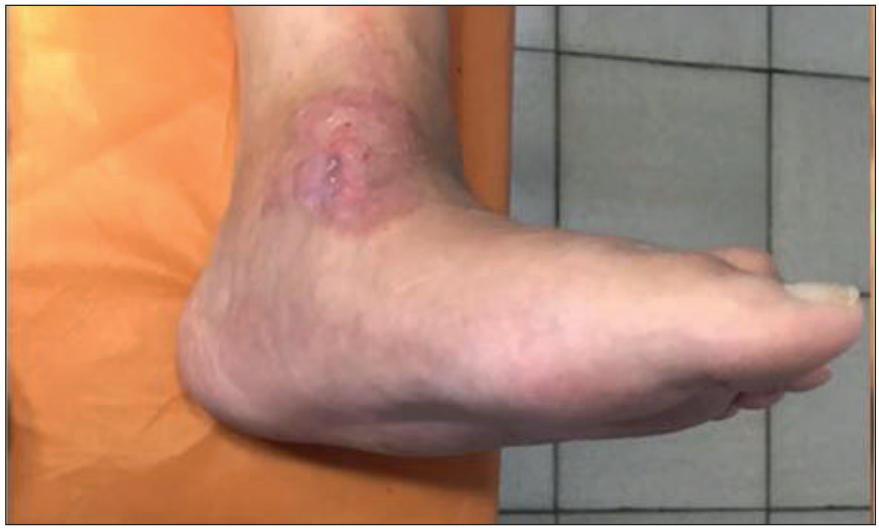


Fig. 5. a), b) Burning shin wound (thermal injury) 5 days of treatment.



Pic. 6. Burning shin wound (thermal injury) 15 days of treatment.

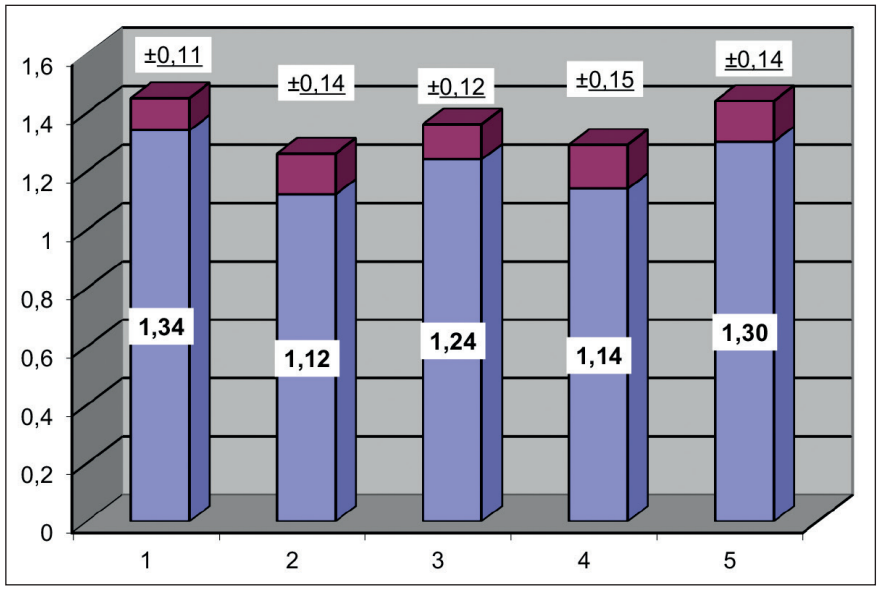


Fig. 7. Dynamics of TX (CD-4) / TC (CD-8) in patients with long-term infected wounds: 1 – healthy; 2 – control group before treatment; 3 – control group after treatment; 4 – the main group before treatment; 5 – the main group after treatment.

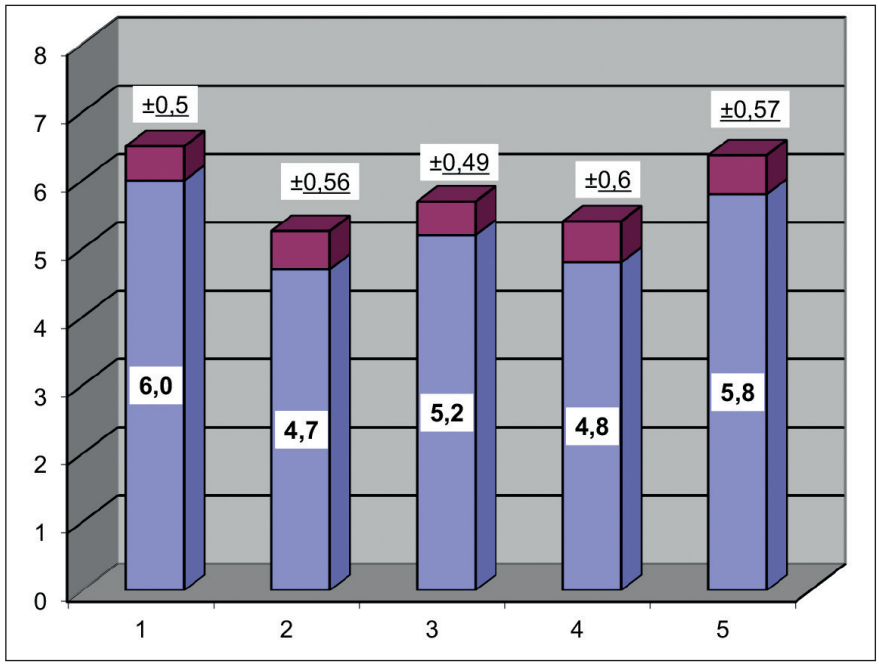


Fig. 8. Dynamics of IgE in patients with long-term infected wounds: 1 – healthy; 2 – control group before treatment; 3 – control group after treatment; 4 – the main group before treatment; 5 – the main group after treatment

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ORIGINAL ARTICLE
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IMMUNOHISTOCHEMICAL AND GENETIC PROGNOSTIC FACTORS OF NEOADJUVANT CHEMORADIOTHERAPY EFFICACY IN PERSONALIZED TREATMENT OF LOCAL ADVANCED RECTAL CANCER

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ABSTRACT

The aim: The aim of our study was to define the factors that can robustly predict a response to neoadjuvant chemoradiotherapy (NCRT) in patients with local advanced rectal cancer (LARC) and prognosis factors of progression free survival (PFS) using molecular (8-oxodGu), immunohystochemical (Ki-67) and genetic (GSTP1 and MTHFR genes polymorphism) markers.

Materials and methods: *GSTP1* and *MTHFR* polymorphisms were studied by real-time PCR on tumour material from 110 patients with LARC. Ki-67 protein expression was assessed using rabbit monoclonal antibodies to Ki-67 (Dako, Denmark) on EnVision™ FLEX detection system (Dako, Denmark). 8-oxodGu level in eluate was measured by spectrophotometry.

Results: Patients from both groups showed significant pathomorphological response to NCRT. It is robust correlation between 8-oxodGu levels in patients' blood and their response to CRT (mrTRG scale) in MG was determined. Oxaliplatin-containing chemotherapy promotes statistically significant decrease of 8-oxodGu levels. With the decrease of Ki-67 protein expression level the probability of tumour relapse increases. It is determined that critical value of Ki-67 protein expression level makes less than 27 and tumour relapse probability in this case makes 50%. Tumour relapse risk in patients with GSTP1 and MTHFR polymorphism is 12.3 and 16.3 times higher than in patients who do not carry such polymorphism, respectively. Combination of GSTP1, MTHFR polymorphisms and Ki-67 protein expression factors determines prognostic probability of tumour relapse within 51-99%.

Conclusions: 8-oxodGu level can serve as independent prognostic factor of NCRT efficacy in patients with LARC. Combination of *GSTP1*, MTHFR genes polymorphism with Ki-67 protein expression decrease enables monitoring and robust prognosis of LARC relapse.

KEY WORDS: neoadjuvant chemoradiotherapy, rectal cancer, prognosis factors.

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INTRODUCTION

Modern tactics for localized rectal cancer treatment is strictly standardized and regulated by International Guidelines (NCCN Guidelines v.2. 2017) [1]. In contrast to localized forms, for LARC that features high probability of relapse and inner organs systemic lesion there still is no finally formulated treatment and diagnostic standard and until very recently even definition by itself was not formulated [2].

To reach proper resectability levels, NCRT is the only solution it was supported by 100% of the experts and certified as the form of Consensus (2013). This procedure enables "stage downgrade" – transformation of locally spread form into localized one, significantly improves postponed treatment results and decreases the number of local relapses. Tumour regression degree after NCRT directly correlates to survival rates and local relapse frequency [3].

Tumour reaction on CRT can vary from complete pathological regression (pCR) to full resistance (according to different authors, regression value makes from 3 to 54 %) [4]. Tumour regression degree after NCRT directly correlates to survival rates and local relapses frequency. Based on randomized clinical trials, it is proved that preliminary course of radiotherapy both fluorouracil and with oxaliplatin promotes tumour regression. Such effect enables definitive organ preserving surgery and leads to local relapses decrease in postsurgical period. NCRT is integral part of combined LARC treatment and can induct partial, significant and total regression of primary tumour with long lasting PFS [5, 6].

Nowadays, the main goal of LARC NCRT is to decrease the relapse development risk in true pelvis by means of maximal reduction of primary tumour size and tumour process stage downgrade. To meet this goal, modified regime of neoadjuvant chemotherapy (NCT) – replacement of fluoropyrimidine monochemotherapy to oxaliplatin polyhemotherapy, looks as a very promising strategy. Yet, growing NCRT interruption frequency resulting from toxicity increase remains to be an issue. Positive impact on general ten-years survival was showed only in one of four multi-institutional randomized research published as for today [7-10]. Nevertheless, in recently published scientific

reports there is no any data about any of chemotherapy agents' advantages. The results of STAR-01, ACCORD 12/0405-Prodige-2, NSAPB P-04, PETACC-6 randomized studies did not demonstrate significant improvement in pathological complete locoregional response and increase in survival rates connected to oxaliplatin addition to the treatment regimen. Moreover, in patients treated with oxaliplatin toxicity increase of 3-4 grade was observed [11-33].

Opposed to this, German CAO/ARO/AIO-04 studies involving integration of oxaliplatin into fluoropyrimidine-based NRT of rectal cancer demonstrated higher pathological complete response (pCR – 17% versus 13%; p = 0.038), higher 3-years PFS (75.9% versus 71.2%; p = 0.03) and no increase of general toxicity in oxaliplatin group [14, 15].

Though, there is no clear data about efficacy of NCRT with multimodal oxaliplatin including treatment in patients with LARC. According to ESMO and NICE recommendations, improved treatment results can be expected in field of personalized approach that considers immediate and postponed relapse risks. The problem of choosing right NCRT should be further investigated taking into account clinical, radiological, pathomorphological data and prognostic markers.

Other very important and insufficiently studied aspects are immunohistochemical and genetic peculiarities of tumour as prognostic factor in RC.

Level of malignancy maturation depends on intensity of tissue and cellular atypism and mitotic activity level. To assess cell proliferation in tumor, series of markers are commonly used. Conventional cell proliferation marker Ki-67 is one of them. This protein is expressed in proliferating cells during G1 phase and is absent in G0 resting cells [16]. This makes Ki-67 clinically significant proliferation marker for prognosis of several cancer types [17-21].

Clinical trials of Z. Pap et al. showed that in adenoma of the large intestine the Ki-67 expression level directly correlates with seriousness of dysplastic changes [22]. Several studies showed that significant Ki-67 expression is mainly connected to lower overal survival rates [23-27], while other studies highlight that high Ki-67 expression correlates with improvement of general survival and better oncological prognosis [28-30].

It is well known that gene polymorphisms participate in colorectal cancer pathogenesis and many chemotherapeutical drugs metabolism. Individual peculiarities of glutathione S-transferase (GSTP1) and methylene-tetra-hydro-folate-reductase (MTHFR) enzymatic activity mediated by gene polymorphism can predict rectal cancer and development of the resistance to NCT (oxaliplatin, 5-fluorouracil, irinotecan). *GSTP1* and *MTHFR* polymorphism detection in patients with RC can be efficient for predicting tumor cell response to chemotherapy and toxicity effects of NCT [31-33].

It is very important that MTHFR participates in antineoplastic agent metabolism (methotrexat, fluorouracil). 5-fluorouracil acts via fluorodeoxyuridine monophosphate and inhibits thymidilate synthase [34]. Drop of MTHFR fermentative activity leads to increase of methylenetetrahydrofolate level and thereby enhances 5-fluorouracil cytotoxicity. *MTHFR* gene polymorphism and efficacy of 5-fluorouracil therapy were estimated in experimental research and clinical trials [35, 36].

It is known that chemical cancerogens such as polycyclic aromatic hydrocarbons and heterocyclic aromatic amines are connected to RC; aromatic amines and some of cytostatins such as platinum products, anthracyclines and steroid hormones are the substrates for GSTP1 enzyme. Substitution of adenine for guanine in position 313 of the 5th exon in *GSTP1* gene leads to isoleucine substitution for valine in position 104 and reduction of its affinity to electrophilic substances [37].

Moreover, polymorphic variants of *GSTP1* and *MTHFR* are the risk factor for gastrointestinal and cardiovasculare toxicity that can be associated with development of resistance to chemotherapy. Genotyping of *MTHFR* C677T polymorphism enables personalization of chemotherapy agents.

In a number of scientific studies, much attention is paid to tumor microenvironment that is one of the key progression factors of tumor resistance to chemotherapy [38]. In addition, further investigations of superoxide radicals and 8-oxoguanine influence as potential markers of RC development are very promising [39-41].

8-oxodGu present in DNA in the absence of reparation can cause cell cycle arrest and apoptosis. Most scientific research indicate the role of oxidative stress in pathogenesis of colon cancer. Chang et al. report that 8-oxodGu content in blood serum can serve as a sensitive biomarker in patients with rectal adenocarcinoma [42] and is considered as a high-informative marker of tumourogenesis [43] and important marker of tumor response to treatment [44].

As a result, this review of combined LARC treatment based on immunohistochemical and genetic prognosis factors implies many complicated unresolved issues that should be addressed in future scientific studies.

THE AIM

Our aim was to define prognostic factors of NCRT efficacy in patients with LARC, namely the prognostic factors of PFS using molecular (8-oxodGu), immunohystochemical (Ki-67) and genetic (*GSTP1* and *MTHFR* genes polymorphism) markers.

MATERIAL AND METHODS

This research is based on the retrospective data analysis from 110 patients, who underwent combined treatment of LARC in Oncocolonoproctology Department of National Cancer Institute from 2016 to 2019 years.

The diagnostic algorithm included estimation of overall patient's status according to ECOG scale, fibrocolonoscopy with biopsy and morphological verification, magnetic resonance imaging (MRI) of thoracic, abdominal and pelvic organs with intravenous contrast, laboratory tests and

electrocardiography. Local staging was conducted using Philips Intera 1,5 T MRI scanner according to MERCURY protocol [45]. All patients confirmed by MRI to be CRM+ were included into the research. It means tumor invasion, metastatic transformation of lymph nodes or extranodal tumor deposits, tumor infiltration of mesorectal soft tissue to a distance of not less than 1 cm from the edge. Accuracy of tumor topography in relation to mesorectal fascia played a critical role in choosing treatment plan, namely administration of NCRT and was the most important reference point for total mesorectal excision (TME). Estimation of tumor regression grade according to MRI is based on mrTRG and RECIST 1.1 (Response Evaluation Criteria in Solid Tumours) criteria.

All patients with LARC considering NCRT method were randomized at a ratio of 1:1 with respect to cTNM-pTNM (T3-4 N0-2 M0, CRM+) index.

Patients from MG undewent radiotherapy with total radiation dose of 50.4 Gy (28 sessions 1.8 Gy each) and polychemotherapy according to CAPOX regimen with oxaliplatin in non-adjuvant regime: capecitabine 200 mg/m^2 in two equal doses, one in the morning and one in the evening, peroral in 30 minutes after meals from day 1 to day 14; oxaliplatin 130 mg/m^2 intravenous on day one of the cycle. The interval between cycles made 21 days.

Patients from CG underwent radiotherapy with total radiation dose of 50.4 Gy (28 sessions 1.8 Gy each) and monochemotherapy based on fluoropyrimidines in non-adjuvant regime: capecitabine 200 mg/m^2 twice a day. The interval between cycles made 5 days. In general, every patient underwent two cycles of chemotherapy. Toxicity effects of NCRT were evaluated according to CTC-NCIC scale (version 4.03, 2010). Eight days after NCRT course its efficacy in all patients was evaluated using MRI according to the protocol mentioned above.

Eight weeks after NCRT course, all patients underwent TME. Histopathological estimation of TME quality was conducted according to P. Quirke method. Post-surgical complications were estimated according to Clavien-Dindo-Strasberg classification within 1-30 days period starting from the day of surgery. All observations are in compliance with Ethics Cometee requirements.

To study *GSTP1* and *MTHFR* genes polymorphism, genomic DNA was extracted from paraffin embedded tumor material using method of NC adsorption on silica-membrane (PureLink Genomic DNA Kits, Invitrogen, USA). DNA concentration was measured using NanoDrop-1000 spectrophotometer (Thermo Scientific, USA). Prior to amplification, DNA concentration was from 2 to 8 ng/μL. *GSTP1* and *MTHFR* genes polymorphisms were studied by allele-specific real-time PCR on 7300/7500 Real-Time PCR Systems (Applied Biosystems, USA). To study *A313G*, *GSTP1* and *C677T MTHFR* single nucleotide polymorphisms TaqMan Probes of MGB-type were used. TaqMan Probes and primer sequences were selected using Primer Express® Software v3.0 (Applied Biosystems, USA) and synthesized by Applied Biosystems, USA. PCR reaction conditions were as following: 50 °C for 2 min, 95 °C for 10 min, followed by 45 cycles at 92 °C for 30 s and 60 °C for 1 min.

The levels and accumulation rates of guanine DNA oxidative damage – 8-oxo-dGuo in neutrophilic blood granulocytes of LARC patients were measured 1 day before surgery [46]. For this purpose, 5 mL of patient's blood from median cubital vein were collected into centrifuge tube with 1 mL of Trilon B. Neutrophils were separated from according to H.T. Lee [42]. The 8-oxo-dGuo was evaluated in eluate on spectrophotometer.

To study Ki-67 expression, obtained material was fixed in buffered 10% formaldehyde, pH 7.4, and embedded in paraffin using Histos-5 (Milestone, Italy). Histological slices 5 μm in depth were obtained using Microm HM325 (Thermo Scientific, USA). The slices were stained with haemotoxilin and eosin and general tumor estimation was conducted. Immunohistochemistry was performed using rabbit monoclonal antibodies to Ki-67 (Dako, Denmark) on EnVision™ FLEX detection system (Dako, Denmark). For antigen retrieval, citric buffer with pH 6.0 was used. Primary antibodies were incubated at room temperature for 30 min., secondary antibodies – for 20 min. at room temperature. After this, slices were additionally stained with haemotoxilin Gill. As a positive control, specimens with determined positive reaction were used. The same procedure without primary antibodies served as a negative control. Quantitative microscopic assessment of NCRT efficacy was done according to I. Miller and S. Payene (2003).

Statistical analysis included descriptive statistics (means as M±m or median – Me, minimal value – min, maximal value – max). Comparison of two independent groups based on one feature was conducted using Mann-Whitney test; comparative assessment of the groups based on variable fraction proportion expressed in nominal or ordinate scale was taken using χ^2 Pearson test. Two variables correlation analysis was based on Spearman's rank correlation coefficient. Logistic regression was used for prognostic marker estimation in LARC treatment efficacy. Null-hypothesis of variables equality was rejected when $p < 0.05$. Data analysis was carried out in IBM SPSS Statistics 22.

RESULTS AND DISCUSSION

Fifty seven patients were randomized into MG and fifty three to CG. Average age of the patients in MG makes 59.3 ± 11.4 years, in CG – 62.5 ± 10.2 years. There were no statistically significant differences in gender, age and anthropometric parameters (body-weight index and total body area) between patients. According to the pathology report, all tumors were adenocarcinomas on varied differentiation stages. Full scale NCRT was conducted in all patients. As a result, the groups were representative.

After the analysis of regression value after NCRT according to MRI (mrTRG and RECIST 1.1 scales), it was determined that tumor response to NCRT was higher in MG – 34 patients (59.7%) and 37 (64.9%) than in CG – 25 (47.2%) and 26 (49.1 %), respectively, however, this difference was statistically insignificant ($p<0.576$; $p<0.329$).

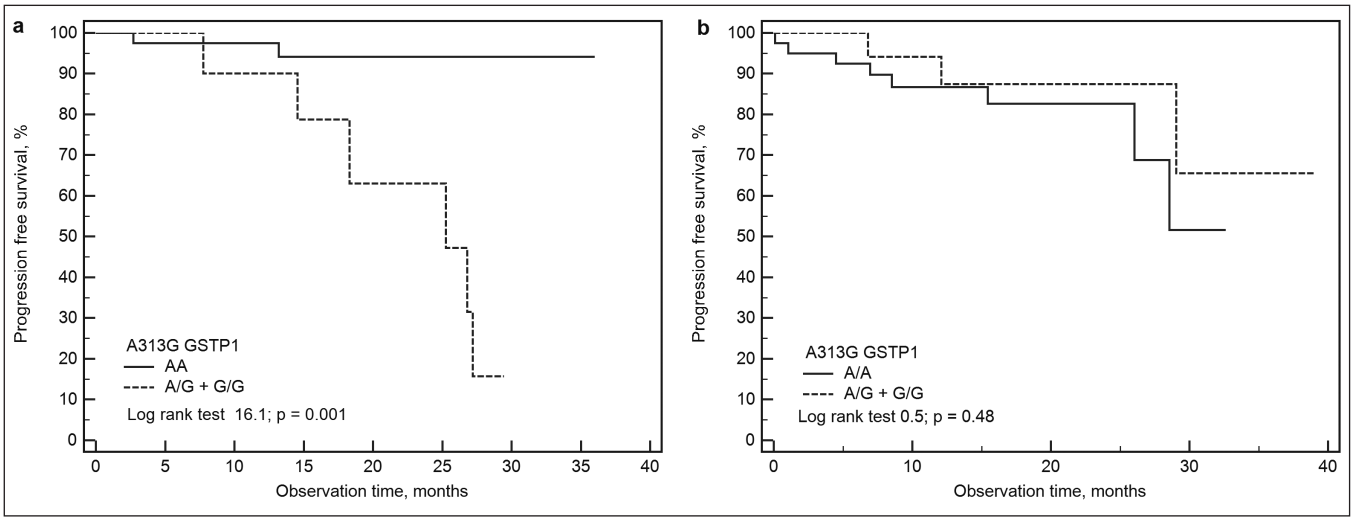

Fig.1. The effect of A313G GSTP1 gene polymorphism on 3-year PFS rate in LARC patients after combined treatment. a – patients received capecitabine as neoadjuvant chemotherapy; b – patients received CAPOX as neoadjuvant chemotherapy.

Significant difference was observed in conversion level of CRM+ to CRM: in MG – 39 (68.4%) and 24 (45.3%) ($p<0.05$).

The estimation of morphometric and topographic anatomy features of tumor regression depending on NCRT scheme showed that in MG the distance between dental line and lower edge of the tumor significantly increased and made 1.1 ± 0.1 cm versus 0.7 ± 0.1 cm in CG ($p <0.005$). The length of the tumor decreased by 28.1 ± 2.1% versus 20.4 ± 2.7% ($p = 0.024$), respectively. Thus, positive effect of NCRT on tumor pathogenesis treatment conducted according to the CAPOX scheme and on the quality of TME was proved.

To make oxaliplatin-bazed NCT more personalized, the level of 8-oxodGu in neutrophils from human blood of LARC patients in both groups was measured. Physiological level of 8-oxo-dGuo makes 0.23±0.04 nmol/mLxmin [19].

The statistical analysis of 8-oxodGu level dependent on chemotherapy scheme prescribed, reviled that application of oxaliplatin-bazed NCT as a part of comprehensive treatment of LARC patients aids the significant decrease of studied marker levels: ($R2 = 0.465$; 95% CI: 0.004 – 0.016, $p<0.0001$). Before NCRT 8-oxodGu level in MG was 3.07±0.08 nmol/mLxmin, in CG – 2.94±0.06 nmol/mLxmin, after it – 1.96±0.04 nmol/mLxmin and 2.72±0.04 nmol/mLxmin ($p<0.001$), respectively. Thus, we can conclude, that 8-oxodGu level can be considered as an independent prognostic marker of NCRT efficacy in LARC patients.

Based on logistic regression analysis, the concentration border level of 8-oxodGu in blood is 2.9 nmol/mLxmin. Based on the data supplied above, lowering of 8-oxodGu concentration is recommended before NCRT according to CAPOX scheme when its level is ≥2.9 nmol/mLxmin.

All patients in both groups underwent surgery 8 weeks after the NCRT. Surgery extent was determined depending on localization and primary tumor expansion rate. All surgical procedures were R0-resections. In MG 37 patients (65%) and in CG 26 patients (47%) underwent laparoscopic surgery. There was no statistically significant difference between groups in terms of surgical intervention structure ($p=0.196$). According to visual estimation, TME performance in both groups was satisfactory. Positional level of tumor location related to anus ($r = 0.431$ in MG and $r = 0.417$ in CG) positively correlated ($p<0.05$) to distal border of rectum resection ($r = 0.510$ and $r = 0.532$), respectively, and inversely correlates to malignancy stage after NCRT ($r = – 0.390$ and $r = – 0.370$) involving circumferential resection margin (CRM+) ($r = – 0.514$ and $r = – 0.522$). Quality of mesorectumectomy was evaluated after Quirke P et al. However, there was no significant difference between groups in terms of differences in surgical intervention method.

In postsurgical period, surgical material was investigated in terms of morphology. Therapeutic pathomorphosis level was evaluated according to parenchymatous tumor tissue percentage, fibrosis and necrosis levels, considering NCRT conducted (CAPOX regimen and capecitabine-based NCRT). Therapeutic pathomorphosis level in MG was 42.0 %, 58.0 % 31.2 %, 51.6 %; in CG: 56.9 %, 43.1 %, 34.3 %, 39.2 %, respectively. Evaluation of prescribed NCRT regimen did not confirm higher efficacy of them in terms of stronger pathological response. In this context we talk about polychemotherapy according to CAPOX scheme versus capecitabine-based monochemotherapy. The fact that we did not observe any significant statistical difference in cumulative frequency of PFS between MG and CG (73.5 ± 8.5 % versus 70.2 ± 2.5 %, $p=0.522$) and correlative general survival (87 ± 7.0 % and 96 ± 2.7 %) proves this. However, according to morphometrical data, there is a statistically significant difference in locoregional therapy response, namely a decrease of parenchymatous tissue and stroma volume 56.9 % and 43.1% in CG resulting from CAPOX scheme NCRT compared to 42 % i 58% in MG ($p<0.001$). This findings lead to the conclusion that none of above described chemotherapy agents has advantages over the

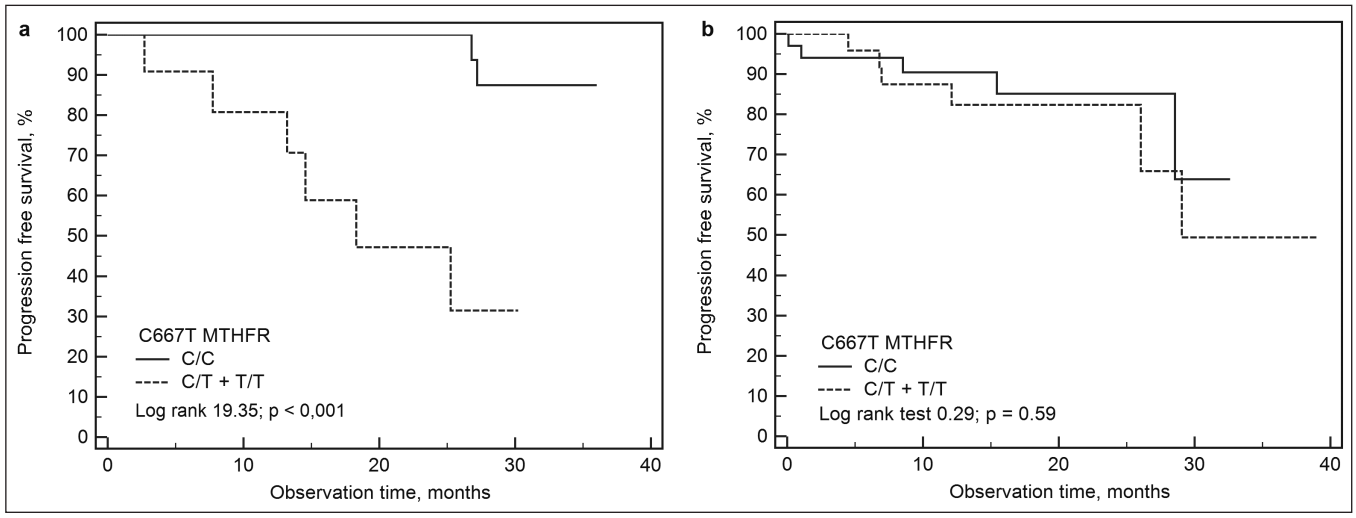

Fig. 2. The effect of C667T MTHFR gene polymorphism on 3-year PFS rate in LARC patients after combined treatment. a – patients received capecitabine as neoadjuvant chemotherapy; b – patients received CAPOX as neoadjuvant chemotherapy.

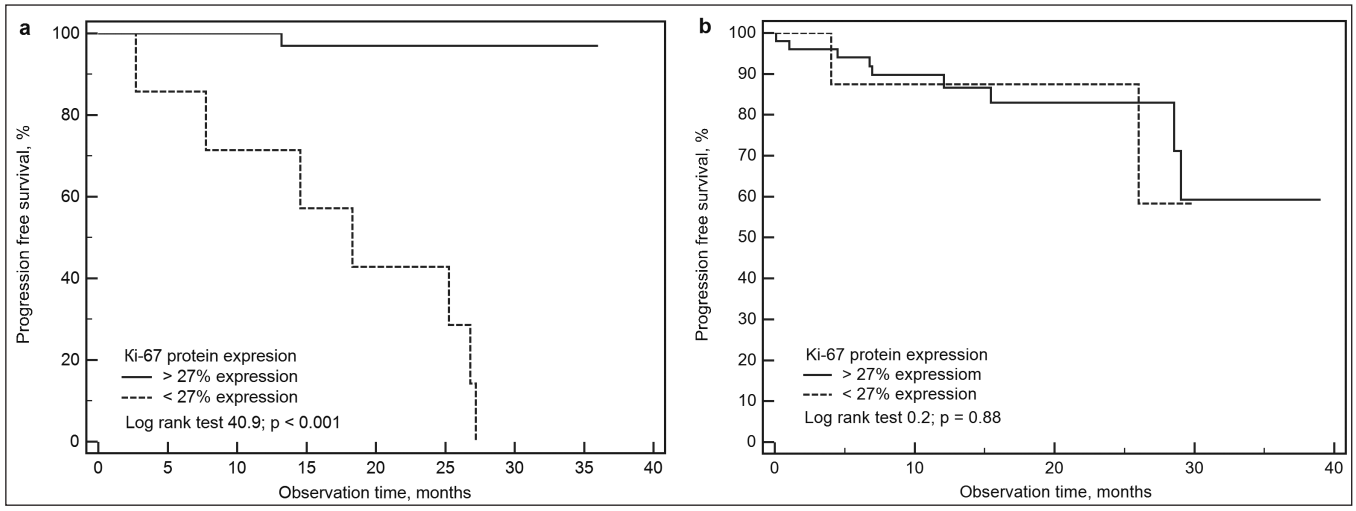

Fig. 3. The effect of Ki-67 protein expression on 3-year PFS rate in LARC patients after combined treatment. a – patients received capecitabine as neoadjuvant chemotherapy; b – patients received CAPOX as neoadjuvant chemotherapy.

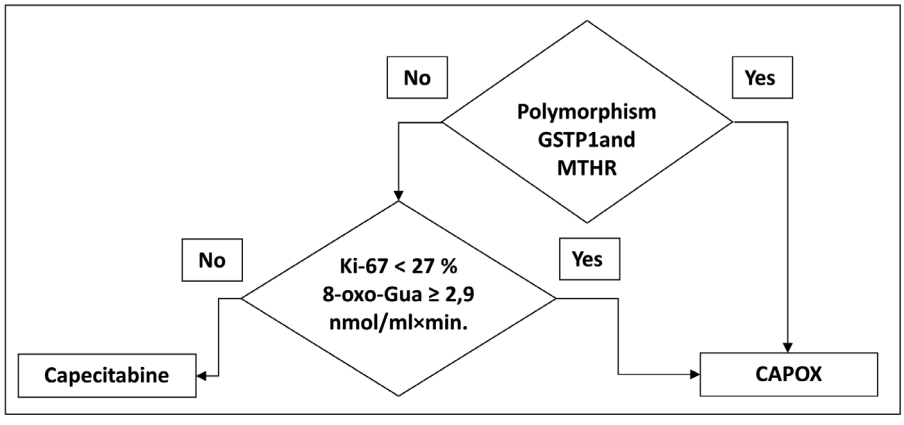

Fig. 4. The personalized algorithm of NCRT application in patients with LARC.

others. A series of international randomized studies listed in introduction came to the same output.

At the same time, to define prognostic factors of rectal cancer course we measured Ki-67 expression levels. The single-factor logistic regression analysis showed that probability of tumor relapse increases when Ki-67 expression level drops. The critical value of Ki-67 expression makes <27% where the probability of tumor relapse chance makes 50%. According to the literature, Ki-67 expression level is a prognostic immunohistochemical biomarker that can predict survival rates decrease in patients with rectal cancer [57]. Comparison of Ki-67 expression level and PFS revealed statistically significant difference in terms of

Table 1. Recurrence prognostic model in patients with local advanced RC (β coefficient value and probability levels for independent prognostic factors)

Value	β*	Vald.**	P
GSTP 1	3,036	4,293	0,038
MTHFR	3,169	4,270	0,039
Ki-67	-0,141	4,520	0,034
Constant	1,397	0,330	0,565

GSTP1	MTHFR	Ki-67 <27%	Recurrence prognostic probability, (%)
+	+	+	= 99
+	+	–	≥ 62
+	–	–	≥ 73
–	+	+	≤ 75
–	+	–	≤ 51
–	–	+	≥ 51

* – β coefficient in logistic regression; ** – significance criterion for β coefficient.

PFS rate in CG. Patients with Ki-67 expression >27% have 27.5 times higher relapse risk compared to patients with Ki-67 expression <27%. In MG, such statistically significant difference was not observed (Fig.1).

Detection of polymorphism in *GSTP1* and *MTHFR* genes in LARC patients is a new innovative research direction. Three-year PFS in patients from CG that carry *GSTP1* and *MTHFR* polymorphism is significantly higher. According to logistic regression analysis, relapse risk in patients who carries *GSTP1* and *MTHFR* polymorphism is 12.3 and 16.3 times higher compared to patients without it, respectively. In CG three-year PFS decreases significantly in patients with A313G *GSTP1* and C667T *MTHFR* polymorphisms compared to the patients without these mutations (p<0.001). In MG, there was no statistically significant difference in relapse-free survival level in patients with or without *GSTP1* and *MTHFR* polymorphism (Fig.2, Fig.3).

Based on such factors as *GSTP1* and MTHFR gene polymorphism and Ki-67 expression level, we developed a mathematical model of relapse in LARC patients (table I). Based on multifactorial logistic regression analysis next formula was generated (Formula 1):

$$p = \frac{1}{1 + e^{-z}} \quad (1)$$

where $Z = \beta_{f1} \times GSTP1 + \beta_{f2} \times MTHFR - \beta_{f3} \times Ki\text{-}67$;
e = 2.72; Z – likelihood of relapse;
β_{f1} – coefficient for GSTP1 factor; β_{f2} – coefficient for MTHFR factor; β_{f3} – coefficient for Ki-67 factor.

It helps estimate relapse risk patient-specifically within 3 years after treatment. Prognostic probability of relapse can be evaluated within 51–99% depending on GSTP1, MTHFR and Ki-67 factors combination.

As a result, personalized algorithm of NCRT selection was developed (Fig.4). In case of *GSTP1* and *MTHFR* polymorphism or their combination as significant prognosis factors, CAPOX scheme NCRT is recommended. If polymorphisms in these genes are absent, Ki-67 (< 27 %) and 8-oxoGu (≥ 2,9 nmol/mLxmin) expression levels should be considered prior to NCRT prescription.

CONCLUSIONS

The analysis of many scientific publications proves that LARC diagnostics and treatment is an essential and very complicated issue in modern clinical oncology. Based on our own knowledge and practical experience in optimization of combined modality LARC treatment considering prognostic factors (8-oxoGu, Ki-67, A313G *GSTP1* and C667T *MTHFR*) we came to clear understanding of personalized NCRT schemes. The algorithm developed from multifactorial system allows to predict and monitoring LARC relapse development. Consequently, it will improve immediate and postponed results of treatment in patients with this complicated heterogenetic oncological disease.

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REVIEW ARTICLE
PRACA POGLĄDOWA

THE RIGHT TO CLONE: SOME ASPECTS OF THE CONTEMPORARY DISCOURSE

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ABSTRACT

The aim: The paper aims to analyze some aspects of the contemporary discourse which concern the determination of the content and specificity of the right to clone. It also outlines the main trends in the development of legal regulation of cloning within international and national law and order.

Materials and methods: Methodologically, this work is based on the system of methods, scientific approaches, techniques and principles with the help of which the realization of the research aim is carried out. There have been applied universal, general scientific and special legal methods.

Conclusions: Regarding the findings of the study it is necessary to note the following. First, if there is a shared negative vision of the feasibility of reproductive cloning in general, which is enshrined in international and national legislation, the need for therapeutic cloning remains an unresolved issue. Secondly, medicine advances and accordingly sees new perspectives and innovative developments in the field of therapeutic activity, in particular, related to the results of therapeutic cloning, which can help in the fight against incurable diseases. Hence, there is the necessity of further research aimed at the improvement of the existing mechanisms for implementing therapeutic cloning, and determining its limits and procedural aspects.

KEY WORDS: human rights of the fourth generation; the right to clone; therapeutic cloning; reproductive cloning

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INTRODUCTION

The 21st century was marked by outstanding advancement in the latest technologies and technological process. Due to the influence of this factor, almost all spheres of human life are modified. Obviously, the legal sphere is not an exception; in particular, the evidence of this, among other things, is the emergence of fundamentally new human rights, which in scientific doctrine, are commonly called fourth-generation rights.

Fourth-generation human rights, including those in the field of health care, are peculiar owing to the fact that they reflect the dynamics of social life and take into account the existing needs of society, creating new opportunities for the realization of human needs that undergo changes over time. However, despite the undoubtedly positive role of such development, these rights remain ambiguous and rather contradictory, sometimes coming into conflict with the shaped perceptions of society about certain norms of behavior, as well as with religious postulates.

The above-mentioned proves that the chosen topic is of vital interest for researchers. However, notwithstanding the considerable number of recent publications in this area, there still remain a number of challenging issues and controversial statements, especially regarding the determination of the content and perception of the right to clone.

THE AIM

The aim of the work is, therefore, to study and analyze some aspects of the contemporary discourse which reflect the current state of understanding and perceiving the appropriateness of the right to clone, characteristics of its nature, as well as to outline the main trends in the development of legal regulation of the sphere of cloning within international and national law and order.

MATERIALS AND METHODS

The methodological basis of this work includes a system of methods, scientific approaches, techniques and principles by means of which the research aim is realized. There have been applied universal, general scientific and special legal methods. Thus, in particular, the methods of analysis, synthesis, induction and deduction made it possible to generalize the obtained knowledge which became the basis of scientific exploration. In addition, due to the usage of the comparative method there have been compared the different points of view presented in the paper, and light has been shed on the specificity of the normative regulation of the investigated issues in certain countries.

REVIEW AND DISCUSSION

First of all, it should be stressed that human rights of the fourth generation in the field of health care are still called somatic, id est, those that are manifested in each person's possibilities to have control over their own bodies. It is believed that one of the first scholars who singled out a separate groupu of somatic rights to is V. I. Kruss. Analyzing V. I. Kruss's works, researchers underscore that somatic rights are difficult to fit

into the existing classifications of human rights, since these rights are aimed at protecting bodily and spiritual integrity; at the same time these rights include an individual's ability to put forward certain personalized requirements to society. However, scholars argue that the nucleus of somatic rights is the right to life and human dignity, freedom of conscience, the right to liberty and personal security, which are fundamental to personal rights [1, p. 24].

As a rule, fourth-generation human rights in health care include the following: cloning; euthanasia; the use of assisted reproductive technologies (artificial insemination, surrogacy); transplantation; gender change; gay marriage and the like.

In the context of our study, the focus is on one of the most controversial rights of the fourth-generation concept in the field of health care, that is, the right to clone. Usually, when it comes to cloning we mean the creation of new living organisms, including humans, in artificial laboratory conditions.

Cloning can be defined as a system of methods used to obtain clones. The term «clone», which etymologically derives from the Greek word «klon» (a branch, sprout, shoot) was introduced in science by the English biologist John Burdon Sanderson Haldane in 1963. In the light of molecular biology it is a system of methods and techniques used for obtaining the cloned DNA or obtaining the genetically identical material in large quantities. There should be distinguished the cloning of genes, organisms, molecular cloning, etc. When cloning genes, individual genes of a cell are isolated and repeatedly copied. This technology can be used to produce a large amount of protein encoded by this gene. This is valuable for pharmacy, because it allows to artificially create protein which is necessary for the body if its natural synthesis is abnormal. In molecular cloning, DNA molecules are reproduced as part of a vector which is a plasmid or phage (DNA cloning).

Cloning of multicellular organisms is the process of transplanting the donor nucleus into the recipient cell, activating this hybrid unless it gets divided, of its development outside the body, and transplanting it into the uterus for further development. It can be embryonic and somatic. In embryonic cloning, the donors of the nuclei are cells of morulas or blastocysts, and in somatic cloning – somatic cells. In comparison with embryonic cloning, somatic cloning is a more recent development [2].

Human cloning is often characterized as the process of making a genetically identical copy of a human. The term is generally used to refer to artificial human cloning, which is the reproduction of human cells and tissues. This does not refer to natural conception (identical twins) [3]. Cloning a human requires the following: 1) a female ova from which its own nucleus is removed; 2) the donor cell to be cloned. The nucleus of this cell is transplanted into the ova; 3) the embryo obtained in this way is transferred into the uterus of the surrogate mother, that is, the woman who has agreed to go through with this pregnancy. A human, born in this way, is a clone. This human inherits entirely the genetic code of a donor (genotype).

However, it should be noted that viewing cloning in this narrow-minded way, from our perspective, much of its content, related to the cloning of human organs and tissues, is lost; the talk is about therapeutic cloning. Reproductive cloning presupposes the creation of a new organism under laboratory conditions wheras therapeutic cloning («cellular reproduction») is the same as reproductive cloning but with an embryonic growth term of up to fourteen days; during the first fourteen days embryonic cells are being formed, further they are able to transform into specific tissue cells of individual organs – a heart, kidneys, a liver, a pancreas, teeth, etc. which are used in medicine for the treatment of many diseases. Such cells of future organs are called «embryonic stem cells» [4, p. 188-189].

Hence, depending on the set goals, there are distinguished two types of cloning. The first type, as the reproduction method, is aimed at reproducing a human or other creatures (reproductive cloning) whereas the second type, cloning for medical purposes (therapeutic cloning), is used for regenerating organs of the same person or producing medicines. The latter does not aim to fully reproduce living beings and methodologically proceeds without the use of a donor uterus.

At the same time, a number of scholars believe that, from a legal point of view, human cloning conflicts with the most important rights of the person, i. e. the right to human dignity and the right to integrity of the person. There is no need to talk about those legal issues that will be caused by the appearance of a human clone. The first issue which arises is related to the fact whether a human clone will be a legal personality, and if so, will his legal personality coincide with the legal personality of the original. An immense legal problem will be the regulation of relationship between the original person and his clone, at least in terms of identification of the person, succession, family relations, etc. [5].

Today, most countries in one form or another have banned cloning. In particular, this applies to Belgium, the United Kingdom, Denmark, Spain, Italy, the Netherlands, Germany, Slovakia, France, Switzerland, Sweden, Japan, which have legislated this issue. Ukraine has also followed this way by adopting the Law «On the Prohibition of Human Reproductive Cloning» in 2004 [6].

Regarding the legislative regulation of this issue in other countries, in Australia and Italy, laws to ban cloning were passed in 2001. The South Korean Parliament, under the influence of the public, passed the law in 1998 which allows cloning a human cell only to fight cancer and other diseases. It should be stressed that most countries signed the Additional Protocol to the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine, on the Prohibition of Cloning a Human Being. They have imposed criminal liability for experimenting in this field: up to 20 years of imprisonment in France, in Germany – 5 years, in Japan – 10 years [7, p. 49].

These days the practice of criminalization of human cloning is actively being in the world. In particular, such

norms are part of the criminal codes of Spain (1995), El Salvador (1997), Colombia (2000), Estonia (2001), Mexico (2002), Moldova (2001), Slovakia (2003). The Criminal Code of France was supplemented by the provision which establishes liability for cloning under the Bioethics Act dated August 6, 2004 [8, p. 151]

Of interest is the situation in Germany, where for a long time there were regulations that severely restricted research dealing with human embryonic stem cells. In particular, its Law «On the Protection of Embryos in Relation to the Import and Use of Human Embryonic Stem Cells» of June 2002 generally prohibited importing and receiving embryonic stem cells (hereinafter referred to as ESCs). However, in terms of import limitations there were introduced some exceptions, in particular, for accomplishing «overarching scientific purposes»: ESC lines could only be imported prpvided they had been obtained (isolated) from embryo-fetal materials only (dead embryos) by 1 January 2002. Nevertheless, on April 11, 2008, the German Bundestag decided to «soften» the time limit set in the previous version, allowing German researchers to import ESCs that had been isolated by May 1, 2007, which certainly expanded their capabilities. Anyway, cloning of human embryos in Germany is forbidden, in particular, in § 6 of the Law of the Federal Republic of Germany «On the Protection of Embryos» which has been in force since 1991. It says: « The one who with his actions creates an embryo that has the same genetic information as another embryo, a fetus, a person alive or dead, shall be punished by a fine or imprisonment for a term up to five years » [9, p. 89].

Italy, Denmark, France and the Netherlands have similar legislation. In Switzerland, the prohibition of cloning is carried out at the constitutional level, in particular, in section «a» of Part 2 of Art. 119 of the 1999 Constitution. It states that all types of cloning and interference with the hereditary material of human gametes and embryos are not allowed [10]. In 2003, the House of Representatives of the United States Congress passed the law that viewed cloning, whose purpose includes reproduction, medical research and treatment, as criminal and could result in imprisonment for a period of 10 years and a fine of $ 1 million. However, in January 2009, criminal liability for therapeutic cloning was abolished [8, p. 151-152].

In 1990 in Great Britain The Human Fertilization and Embriology Act was adopted; according to it, « the fusion of cell nuclei of the human embryo with nuclei which were isolated from cells of another person's tissue, of an embryo or foetus, is forbidden ». It is based on the Governmental Commission's report on Ethical Issues in Embryology. The law prohibited human cloning, «if cells removed from embryonic tissues were used for this purpose». In cases when the donor was an adult organism, this prohibition did not apply.

Already in 2000, the British Parliament cancelled the legal restrictions on cloning. In December of that year, the House of Commons approved a bill that allows the use of cloned human embryos for scientific purposes. And in January 2001, 212 members of the House of Lords voted in favor of it (92 members voted against it). However, in June, the British Royal Society advocated the prohibition of human cloning, except for the cloning of human cells for therapeutic purposes [7, p. 49].

In this context, it is worth noting that in some countries (Australia, Belgium, Italy, Colombia, Mexico, New Zealand, the Netherlands, Romania, France, Sweden, etc.), despite the existing ban on reproductive cloning therapeutic cloning is allowed.

In addition to the prohibition at the national level in some countries, prohibition norms also act at the level of the international community. For example, in 1997 the Universal Declaration on the Human Genome and Human Rights was adopted, which in Art. 11 enshrined the impossibility of cloning as a practice which is contrary to human dignity [11].

In 1997, there was adopted the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the application of Biology and Medicine: the Convention on Human Rights and Biomedicine. According to this document, any interventions undertaken to modify the human genome can be carried out only for prophylactic, diagnostic or therapeutic purposes, and only if they are not aimed at making any alteration in the genome of the offspring. However, it is stated that the cultivation of human embryos for research purposes is prohibited [12].

Therefore, in 1998, in the light of scientific advances in the field of mammalian cloning and fears that human cloning could become a viable opportunity through the development of bioethics, medicine and new technologies, a number of European countries signed an Additional Protocol to the Convention on the Protection of Human Rights and Human Dignity with regard to the Application of Biology and Medicine, on the Prohibition of Cloning of Human Beings. This document prohibits any interference with the purpose of creating a human being that is genetically identical to another human being [13].

It must be emphasized that in 2005, the UN General Assembly adopted the Declaration on Human Cloning, stating that cloning for the purpose of reproducing a human being is contrary to human dignity and should not be allowed by UN member states, which are to take steps to ban human cloning as quickly as possible at the national level and take all possible measures to prevent it [14].

At the European Union level, the issue of cloning is raised in the Charter of Fundamental Rights of the European Union dated December 7, 2000. In this act, cloning is discussed in the context of Art. 3 which concerns the right to personal inviolability which, according to the authors of the document, is protected, in particular by prohibiting reproductive cloning [15].

Admittedly, the European Court of Human Rights (ECHR) considers it inadmissible to create embryos artificially for the purpose of their further use for scientific purposes [16]. Particularly, this is observed in the ECHR's case of «Parrillo versus Italy», which forbade the com-

plainant to donate his embryos obtained by fertilization for scientific purposes, with the emphasis that embryos cannot be objects of property which can be freely disposed of by the person [17].

The European Court of Justice in its decision related to « Oliver Brüstle v. Greenpeace » case dated 2011, also referred to the prohibition of patenting the results of the research that had been obtained by destroying a human embryo or using it as the source material [18, p. 139].

It should be underscored that the content of the documents analyzed above manifests and proves that the prohibition is imposed only on reproductive cloning, since therapeutic cloning does not include any characteristics indicated in these acts. That is why the issue of feasibility of the latter remains open. The urgency of its solving is reinforced by the fact that, at the national level, in some countries, as noted above, therapeutic cloning is permitted.

If we generalize the positions of proponents of the prohibition of cloning, they suggest the following arguments: 1) cloning violates human dignity, reduces human life to the level of «biological material»; 2) it separates the sphere of childbearing from the true human context of the matrimonial act; 3) demonstrates the lack of respect for human embryos that will be destroyed so that reproduction of this type can occur successfully (in the case with cloning Dolly the sheep there were made 277 attempts, 8 of them were successful and brought to the embryo develepment, as a result, only one sheep was born; 4) cloning is a radical manipulation of the human reproduction, in which personal relationships between parents and children are broken and this can lead to the disappearance of the concept of family and family relationships; 5) cloning is inadmissible taking into account the cloned person's dignity. Everyone has the right to his own uniqueness. The human body and genotype are also an integral part of dignity and uniqueness, whereas a cloned human being is always a «copy» of someone else, which can lead to the loss of human identity and to the feeling of inferiority; 6) cloning creates the danger of social manipulation in the light of eugenics, the choice of «genetically better» people; 7) producing «clones» of living persons solely as a source for organ transplantation makes one view the person merely as an object of use, which is completely unacceptable from the point of view of Christian personalism [19, p. 8].

However, such a perception of the right to clone is a rather narrow-minded approach, which contradicts the very essence of science on the one hand, and on the other hand, it comes to assessing the nature of reproductive cloning. In general, accepting the researchers' vision of this type of cloning and agreeing to it, we consider it necessary to dwell upon possible useful results of the approbation of therapeutic cloning. First of all, ther should be mentioned the arguments of researchers who advocate for the right to clone in general. As a matter of fact, they defend the personal right of everyone to reproduce, to continue the bloodline, which is an integral part of the person's autonomy (along with such rights as the right to contraception, in vitro fertilization, artificial insemination, etc.). The technology of somatic cell nucleus transfer (cloning), in their opinion, is just one of the varieties of the production mechanism. They are convinced that the ban on cloning contradicts the principle of freedom of scientific research. The laureates of the International Academy of Humanism, the moral and ethical issues generated by cloning are not bigger than those people have already faced (nuclear energy, recombinant DNA or computer modeling) – they are just new [20, p. 73].

As for therapeutic cloning, when it comes to cloning of cells and tissues of living organisms with the use of modern molecular-genetic methods, we believe that humanity receives and will receive the benefits of the application of such technologies. These benefits in no way violate or diminish human dignity, because the outcomes of these activities can improve the functioning of science, medicine, agriculture and more. Particularly significant is the aspect related to therapeutic activity, since it is impossible to deny the importance of therapeutic cloning for overcoming serious diseases such as cancer, diabetes, Parkinson's disease, Alzheimer's disease and others.

The fact which is worth mentioning is that this year British researchers have succeeded in creating artificial nerve cells that can be used to treat humans in the future. These are tiny chips made of silicon, to which the researchers managed to transfer the electrical properties of brain cells. They reproduced two types of neurons: nerve cells from the hippocampus – the part of the brain responsible for memory and thee cells involved in regulating respiration. The researchers stress that they want to involve artificial nerve cells in the treatment of diseases which cause the degeneration and death of neurons, for example, Alzheimer's disease or cardiac failure.

The researchers from Israel managed to print a real heart on a 3D printer. Human fat cells were used as the material for producing a heart; they were transformed into stem cells of the cardiovascular muscle and connected with connective tissue. After conducting the research, the heart can be used in transplantology. The developers think that in the next few years there will be an opportunity to create any organ for transplantation, taking into account the peculiarities of each patient [21].

Thus, it is obvious that scholars are trying to develop mechanisms for the «restoration» or «replacement» of human organs and tissues in order to preserve and extend human lifespan, as well as to use the biological materials, obtained in this way, as therapeutic agents and medicines. This mechanism, which is an alternative to the current practice of organ and tissue transplantation from a donor (a living or dead person) to another person (a recipient), makes it possible to completely eliminate the criminal «component» and to significantly increase the likelihood of engraftment of organ and tissue obtained as a result of self-transplantation. Currently, there are positive results of therapeutic cloning of cells taken from a patient who requires the implantation of a particular organ or tissue, as well as the use of the technology of obtaining stem

cells from umbilical cord blood. Experimental cloning of organs or tissues for self-transplantation is also carried out, during which a cell, taken from a particular person, allows growing an organ or tissue for this person. In experts' opinion, such transplantation will never lead to incompatibility, so it will not require the use of special drugs that prevent the rejection of transplanted organs or tissues whose DNA is identical, and will not cause any side effects [22].

Speaking about the cloning situation in Ukraine, as it has been previously stated, in 2004 the Law «On Prohibition of Human Reproductive Cloning» was approved. However, therapeutic cloning remains unaddressed. According to such experts in the field of medical law as Prof. S. H. Stetsenko, Prof. V. Yu. Stetsenko and Assist. Prof. I. Ya. Seniuta, the necessity of introducing therapeutic cloning in Ukraine is indisputable, but they advocate the expediency of introducing therapeutic cloning on the territory of Ukraine gradually (in stages), that is, initially for a certain period (determined not by time frames, but by the readiness of society and the state for this process). This will enable to prevent abuse, scientific failures, violation of ethical and moral principles. We shall be able to talk about the possibility and expediency of permitting therapeutic cloning in Ukraine only with time, when a proper legal framework has been established and all the necessary authorities have been created (for example, the ethical and legal committee consisting of independent experts) to sustain this process through the development and implementation of governmental programs concerning the study of this issue, analysis of scientific, practical , experimental experience of foreign countries, taking into account the opinions of researchers who work in different spheres, as well as the public opinion, [10].

CONCLUSIONS

Thus, the issues of the fourth generation in today's realities are becoming more global and require thorough doctrinal approaches to the study and analysis of their nature in general and their separate components in particular. As for the right to clone, which became the focus of this scientific exploration, we consider it necessary to highlight the following. First, talking about the feasibility of reproductive cloning, generally, there is the common opinion, which is enshrined in national and international legal acts, whereas when it comes to therapeutic cloning, the opinion on its need is either largely defended or unclear (as, for instance, in Ukraine).

Second, there is no denying the fact that medicine advances and accordingly sees new perspectives and new developments in the field of therapeutic activity, in particular, in connection with the results of therapeutic cloning, which can help to combat incurable diseases. It can be anticipated that the development of therapeutic cloning is inevitable and, definitely, needed to improve the living conditions of present and future generations. That is why the research aimed at improving the existing mechanisms for conducting therapeutic cloning, determining its boundaries and procedural aspects should be continued and deepened. This, in turn, will help to create and provide the proper conditions for the realization of individuals' right to clone if there are needs related to threats to their lives and health.

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REVIEW ARTICLE
PRACA POGLĄDOWA

PERINATAL AND INFANT MORTALITY IN THE TRANSCARPATHIAN REGION AND UKRAINE AGAINST THE BACKGROUND OF THE EUROPEAN UNION AND THE WORLD: A COMPARATIVE ANALYSIS AND POSSIBLE PROBLEMS

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ABSTRACT

The aim of this article was to analyze the dynamics of perinatal mortality and mortality up to 1 year in the Transcarpathian region and Ukraine in comparison with other countries of Europe and the world.

Materials and methods: The study is based on data from the Transcarpathian Regional Medical Information and Analytical Center, the Center for Medical Statistics of the Ministry of Health of Ukraine, the European database «Health for All» and the Center for Research of Health Services at the University of Kent, Kiev Economic Institute of the Kiev School of Economics (July 2017).

Review: In 2016, mortality under the age of 1 year in Ukraine amounted to 7.4 per 1000 live births, which is 13.5% lower than the same indicator in 2012 (8.4). According to perinatal mortality, in Ukraine this indicator has a level of 8.59 ‰, while the average in the EU countries does not exceed 6.01 ‰.

Conclusions: Perinatal and mortality rates up to 1 year in the Transcarpathian region, as in Ukraine as a whole, are an order of magnitude higher than the European average. The same negative trend in the survival of newborns in Transcarpathia and in Ukraine.

KEY WORDS: perinatal mortality, infant mortality, regionalization, perinatal care

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INTRODUCTION

The population of any country today is experiencing a difficult period in terms of conditions and lifestyle. Daily stresses, overstrain, an increase in the number of cases of self-medication through uncontrolled hyperinformatization of society, ecology, lifestyle have a direct impact on the health status of the population. In such conditions, the issue of restoring the population, preserving the life of each newborn with a parallel increase in the quality of life not only of the child population, but of the whole society becomes more and more urgent. In this format, the problem of preserving the life and health of each child has a high medical and social significance and determines the well-being and national security of the country [1].

One of the main determinants of preserving the life and health of each child is the state of health of the parents, including the mother, the pregnant woman, the physiological maturity of the fetus, the adequacy of management and the state of the course of labor. Given the importance of the infant mortality rate for civilizational development, this area of research has been and remains relevant both in Ukraine and around the world. This problem was dedicated to the work of the classics of Ukrainian demography and sanitary statistics O.V. Korchak-Chepurkovsky and Yu.O. Korchak-Chepurkovsky, M. Ptukha, S.A. Tomilin, A. P. Khomenko [2]. To assess the state of health of the child population, and in parallel – the quality of medical care for mothers and children, remains a complex multifactorial problem. One of the key indicators that can correctly and efficiently illuminate the actual state of these issues remains mortality in the perinatal period.

Now there are a huge number of sources from which those related to perinatal mortality are of significant interest [3, 4, 5, 6], since this segment plays a decisive role in shaping the overall infant mortality rate in developed countries and is much more difficult to influence by policy in health than, say, infectious diseases [2].

In Ukraine, as well as around the world, over the past decade, thanks to the implementation of World Health Organization (WHO) strategies and programs, focused mainly on reproductive health priorities, it has been possible to significantly reduce maternal, birth and perinatal losses. This was also facilitated by the reorientation of healthcare institutions to a scientifically-based area of activity, evidence in practice, and certain aspects of the regionalization of medical care for these segments of the population.

Even taking into account the positive tendency for these indicators to change, the level of maternal and child health in our country remains one of the least acceptable in comparison with other European Union (EU) countries and the

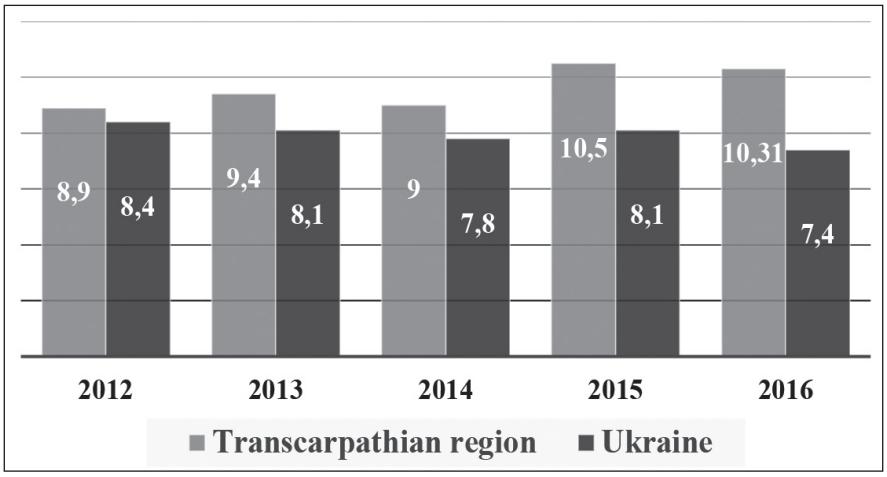

Figure. 1. Mortality rates for children under 1 year old for 2012-2016 in the Transcarpathian region and Ukraine (per 1000 live births).

world as a whole. Today, in developed EU countries, the main methods for optimizing indicators of maternal health, perinatal pathology and the course of childbirth remain the use of modern information technology and scientific achievements, principles of evidence, continuity in perinatal medical practice, which is embodied in the introduction of models of regionalization of perinatal care (RPC). According to reliable studies, the organization of a three-level system of perinatal care (PC), the modernization of technical equipment in Japan, the USA and Western Europe allowed several times to reduce maternal and fetal-baby losses, the incidence and mortality rate of newborns and young children [1].

THE AIM

The aim of this article was to analyze and evaluate the dynamics of perinatal mortality and mortality rates up to 1 year in the Transcarpathian region and Ukraine as a whole compared with other countries of Europe and the world; put forward hypotheses about the possible causes of this situation.

MATERIALS AND METHODS

The study was based on data from the Transcarpathian Regional Medical Information and Analytical Center, the Center for Medical Statistics of the Ministry of Health of Ukraine, the European database "Health for All" and the Center for Health Services Studies University of Kent, Kyiv Economics Institute Kyiv School of Economics (July 2017). Statistical data processing and a graphical representation of the results were performed using standard Microsoft Office Word, Exel 2010 software services, as well as STATISTIKA, V.10.0.

REVIEW

For the period 2012-2016 Infant mortality rates in Ukraine had a positive trend. In 2016, mortality under 1 year in Ukraine amounted to 7.4 per 1000 live births, which is 13.5% lower than the same indicator in 2012 (8.4). Unlike Ukraine, the Transcarpathian region by a similar indicator has a level above the national average and is moving upward. Thus, the infant mortality rate in Transcarpathia in 2016 increased by 15.8% compared to 2012 (Figure. 1).

A similar indicator in the EU and the world had a diverse trend, but was significantly lower than in Ukraine as a whole and Transcarpathia in particular. So, as of 2016, the mortality rate of children under 1 year of age in the EU was 3.78 per 1,000 live births. In the dynamics of 2012-2016 This indicator in the EU countries decreased by 5.8%. Similar indicators in other countries as of 2016 ranged from 1.4 ‰ in Iceland and Luxembourg, to 9.8 ‰ in Romania and 12.9 ‰ in the Republic of Moldova, presented in Table I.

If we compare the rate of perinatal mortality in Ukraine and similar in the EU and the world, then in our country, with a positive trend, this indicator changes from 9.78 ‰ in 2012. Up to 8.59 ‰ in 2016 (-13.85%), there is still a predominance of its average perinatal mortality rate in the EU countries (6.09 in 2012 and 6.01 by 2016) – (see Table II).

According to the WHO, the level of perinatal mortality should be due to its high level among those born with low body weight (less than 1500 g), which, in turn, is due to its high level among those born with very low body weight (500-999 g) [7]. Compared with other EU countries and the world, Ukraine maintains a relatively high level of perinatal mortality in children with low and normal body weight (≥ 1000 g), despite the positive dynamics for the period 2012-2016. (Table III).

One of the goals of ensuring a healthy lifestyle and promoting well-being for all at any age, among the goals of sustainable development, is determined until 2030 "to put an end to mortality, which can be prevented, for newborns and children under 5 years of age", to reduce the maternal mortality rate, to ensure general access to services on sexual and reproductive health, including family planning services, information and education, and integrating reproductive health into national strategies and programs [7, 8].

These tasks in Ukraine, unfortunately, are not fully implemented today, in particular in certain regions. This clearly demonstrates the survival rate of newborns with low body weight (1000-1499 g) in the Transcarpathian region compared with other regions of the country (Figure. 2).

Table. 1. Dynamics of child mortality rates up to 1 year in the EU and the world for the period 2012-2016 (per 1000 live births).

Year / Country	2012	2013	2014	2015	2016
The Netherlands	3,5	3,3	3,2	3,2	3,15
Norway	2,3	2,3	2,2	2,0	2,0
Poland	4,5	4,5	4,45	4,3	4,2
Portugal	3,1	3,1	3,1	2,9	2,8
Romania	10,9	10,5	10,1	9,85	9,8
Russia	9,1	8,6	8,5	8,3	8,2
Moldova	13,8	13,3	13,2	13,0	12,9
Slovakia	6,3	6	5,9	5,7	5,6
Spain	3,7	3,6	3,5	3,3	3,3
Sweden	2,4	2,4	2,3	2,1	2,1
Switzerland	3,7	3,6	3,4	3,2	3,2
England	4	3,9	3,8	3,6	3,5
Luxembourg	1,7	1,6	1,55	1,5	1,4
Iceland	1,7	1,6	1,6	1,5	1,4
Ukraine	8,4	8,1	7,8	8,1	7,4
EU	4	3,9	3,85	3,8	3,78

Table. II. Dynamics of perinatal mortality in the EU and the world for the period 2012-2016 (per 1000 births alive and dead).

Year / Country	2012	2013	2014	2015	2016
Austria	2.96	3.13	2.88	2.9	2.85
Belorussia	2.94	2.95	2.83	2.92	2.93
Belgium	4.24	3.78	4.03	4.12	4.09
Bulgaria	10.95	10.29	10.41	9.09	9.2
Czech Republic	3.63	2.93	3.1	2.95	3.23
France	11.58	11.71	11.78	11.81	11.79
Georgia	11.63	10.89	9.1	9.24	9.05
Germany	5.29	5.45	5.45	5.59	5.42
Hungary	4.03	3.85	4.23	4.14	3.94
Iceland	2.23	1.17	2.78	2.62	2.51
Luxembourg	4.66	3.98	2.88	4.53	4.24
Holland	3.66	3.76	3.69	3.55	3.42
Poland	4.14	3.74	3.45	4.96	4.51
Portugal	3.35	4.16	5.14	4.43	4.21
Moldova	8.35	8.19	8.24	7.65	7.78
Romania	6.22	6.49	6.62	6.04	6.24
Russia	6.92	6.58	6.24	5.77	5.51
Slovakia	4.7	4.16	4.58	4.79	4.42
Spain	4.64	4.59	4.57	4.34	4.29
Sweden	3.17	3.42	3.49	3.12	3.04
Switzerland	2.79	3.02	2.89	2.79	2.58
Ukraine	9.78	9.1	9.26	9.1	8.59
EU	6.09	6.09	5.97	6.04	6.01

Table. III. Dynamics of perinatal mortality in children with low and normal weight (≥1000 g) in Ukraine and the EU and the world for the period 2012-2016

Year / Country	2012	2013	2014	2015	2016
Austria	2.96	3.13	2.88	2.9	2.92
Belorussia	2.94	2.95	2.83	2.92	2.93
Belgium	4.24	3.78	4.03	3.87	3.79
Czech Republic	3.63	2.93	3.1	2.95	2.9
Finland	2.63	2.19	2.71	2.63	2.51
Georgia	11.63	10.89	9.1	9.24	9.15
Hungary	4.03	3.85	4.23	4.14	3.97
Iceland	2.23	1.17	2.78	2.45	2.21
Luxembourg	4.66	3.98	2.88	4.53	4.18
Holland	3.66	3.76	3.69	3.55	3.41
Poland	4.14	3.74	3.45	3.32	3.18
Moldova	8.35	8.19	8.24	7.65	7.48
Romania	6.22	6.49	6.62	6.04	6.19
Russia	6.92	6.58	6.24	5.77	5.46
Slovakia	4.7	4.16	4.58	4.23	4.12
Sweden	3.17	3.42	3.49	3.12	3.26
Switzerland	2.79	3.02	2.89	2.79	2.68
Ukraine	7.58	8.12	7.37	6.63	6.14

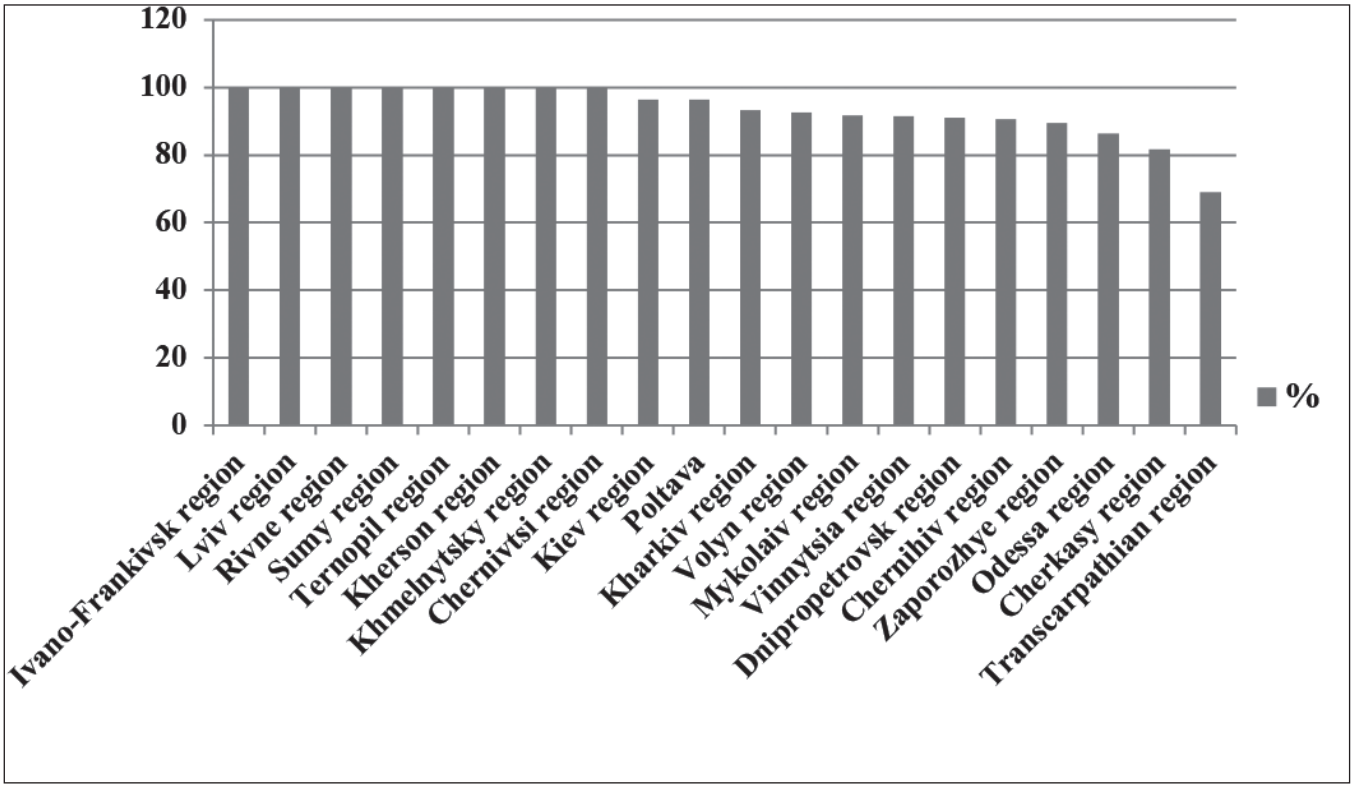

Figure. 2. The survival rate of newborns with low body weight (1000-1499 g) in the Transcarpathian region and other regions of Ukraine in 2014

As can be seen from the diagram, the Transcarpathian region in 2014 had a low survival rate for this cohort of newborns, and this despite the fact that, according to the Center for State Statistics of the Ministry of Health of Ukraine, Transcarpathia is one of the most positive demographic indicators, in particular the birth rate, among other regions and in comparison with the national average.

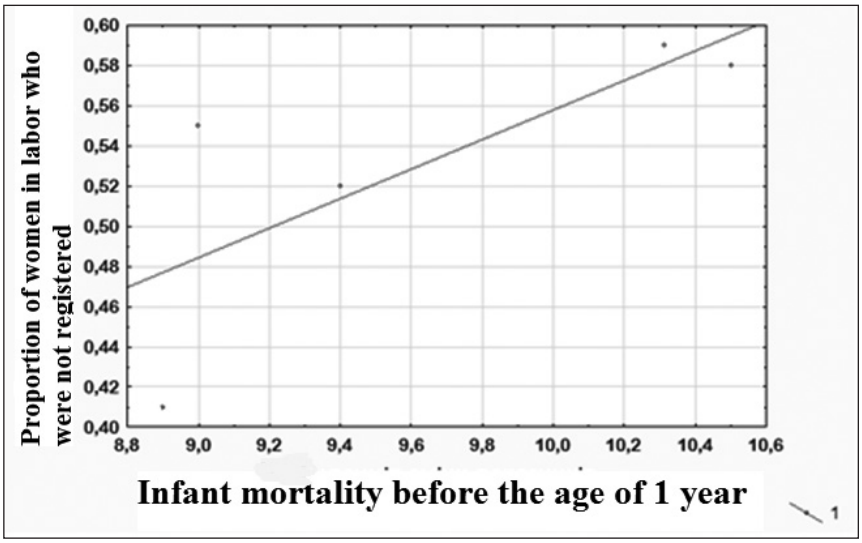

Figure. 3. Regression model of the dependence of infant mortality rates and the proportion of women who gave birth without ever attending a women's consultation (2016).

According to WHO, the main reasons for the high level of perinatal mortality and infant mortality in Ukraine, as in most European countries, are not only the deficiencies of the PC system, but also the attitude of future mothers to their health and the health of the unborn child. If in developed EU countries the indicator of the proportion of women who have given birth without ever having visited the antenatal clinic is practically zero, then in Ukraine this indicator ranges from 0.3-1%. In the Transcarpathian region, in particular, this coefficient with a positive trend of changes for the period 2012-2016. Still holding high. When constructing a regression model between the mortality rates of infants and the proportion of women who gave birth in Transcarpathia, never having visited a antenatal clinic, we obtained reliable data on the dependence of these two indicators (Figure. 3).

Thus, the above fact confirms that the system of perinatal care in the region requires regionalization in order to save those lives that we can save with the adequate functioning of the internal and external sides of the RPC system.

Minimization of the weaknesses of the internal environment of the RPC should be aimed at completing the development of regional regulatory legal acts on the creation of a three-level system of perinatal care in the region and its implementation by creating a level III regional perinatal center in the Transcarpathian region; the creation of a level II regional perinatal center, viewing programs for pre- and postgraduate training of medical workers providing perinatal care of I-III levels; increasing the availability of highly specialized perinatal care in rural areas, including in mountainous and remote areas of all regions, for pregnant women with diabetes; development and implementation of a system of planning, medical observation, early intervention and palliative care for young children with chronic perinatal pathology [7, 8].

The prospect of further research is the study and analysis of the structure and rates of perinatal mortality in the Transcarpathian region and Ukraine in comparison with other EU countries and the world in order to verify "weaknesses" and deficiencies in the existing PC system.

DISCUSSION

The key to the successful implementation of the RPC system in any region with its subsequent effective functioning is not only the development of functional models, although without them in any way, but their adaptation to a specific situation with maximum fit and the solution of all related issues on the way of their implementation in practical activities. For the most adequate prediction of the efficiency of the RPC system, it is necessary to determine the weak link of the existing system. According to the latest WHO recommendations, indicators that can give the most objective information about the work of an individual PC link are: mortality of infants up to 1 year old and its structure, perinatal mortality and its structure, indicators of the morbidity of the child population with an analysis of the structure, and indicators of the state of health of the maternal population . The main ones listed are precisely mortality up to 1 year (infant mortality) and perinatal mortality.

This article highlights part of a dissertation research. The results of our study objectively revealed the urgency of the problem in the Transcarpathian region and in Ukraine as a whole compared with the countries of the European region and the world, the data are reliable and exhaustive. It is proved that at the primary and secondary levels of organization of perinatal care for the population, there are serious shortcomings, which ultimately lead to a deterioration in the health status of the child population. The possible causes of this problem described by us require scientific justification and evidence.

The prospect of this study is the study of the impact on the level of perinatal mortality and infant mortality problems in the staffing of this link in medical care. As you know, in the Transcarpathian region and Ukraine today there is a very active emigration of highly qualified personnel outside the country. There is also a problem with financial and logistical support, as well as with the quality of training of young specialists. The purpose of further research will be to identify a possible relationship between these factors and the development of a perfect model for

the functioning of perinatal services in the region and in Ukraine as a whole.

CONCLUSIONS

1. The infant mortality rate (up to 1 year) in the Transcarpathian region is higher than the national average, and significantly higher than the average European.
2. The indicators of perinatal mortality, both general and in certain weight categories, born alive and dead, in our country are relatively high. Separately, this concerns the fact that children with low and normal body weight at birth still die too often in Ukraine, which is unacceptable from the point of view of WHO and developed EU countries, which minimized these losses decades ago.
3. In the study area, while maintaining relatively high birth rates and natural growth, the lowest survival rate for children with low body weight (1000-1499 g) is observed, which indicates significant shortcomings in the work of individual parts of the system of medical care for mother and child. These facts are confirmed by the relationship between the mortality rate of infants and the proportion of women who have given birth without having ever visited a antenatal clinic (or not registered).

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REVIEW ARTICLE
PRACA POGLĄDOWA

PRIORITIES OF ANTI-HYPERGLYCAEMIC DRUG THERAPY IN PATIENTS WITH TYPE 2 DIABETES AND HEART FAILURE

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ABSTRACT

The aim is to explore the possibilities of improving the effectiveness in preventing cardiovascular diseases and heart failure using sodium-glucose co-transporter 2 inhibitors.

Materials and methods: The analysis of the existing clinical and experimental data on the effect of sodium-glucose co-transporter 2 (SGLT-2) inhibitors on the cardiovascular system, the condition of kidneys, cardiovascular risk factors.

Review: SGLT-2 inhibitors are the first class of glucose-lowering agents in large-scale studies (EMPA-REG OUTCOME, CANVAS, CVD-REAL, CVD-REAL2) which have demonstrated the ability to improve cardiorenal outcomes and reduce the risk of hospitalization with heart failure in patients with diabetes. In addition to hypoglycaemic action, SGLT-2 inhibitors show a number of pleiotropic effects, which are potentially capable of reducing cardiovascular risk: diuretic effect, decrease in: blood pressure, arterial wall stiffness, waist and body weight, expression of albuminuria, etc. The use of drugs of this class opens great prospects not only in terms of glycaemic control, but also in the prevention of cardiovascular complications of diabetes.

Conclusions: 1. When choosing glucose-lowering agents in patients with type 2 diabetes, it is necessary to take into account their impact on the risk of development and the course of heart failure.

2. SGLT-2 inhibitors ought to be considered as a preferred method of treatment for type 2 diabetes in patients with heart failure or with a risk of heart failure that meets the latest recommendations of the European and American Diabetes Association.

KEY WORDS: type 2 diabetes; heart failure, sodium-glucose co-transporter 2 inhibitors

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INTRODUCTION

Nowadays cardiovascular pathology and diabetes represent a significant medical and social problem due to the serious consequences for health, work efficiency, life expectancy and life quality of patients [1]. Diabetes mellitus is an independent risk factor for cardiovascular diseases. At the same time, cardiovascular complications are the major cause of death in patients with type 2 diabetes [2]. At the same time, the risk of developing heart failure in patients with type 2 diabetes increases 2-4 times [3, 4]. This leads to a significant increase in the frequency of hospitalization and a pronounced deterioration in prognosis: 5-year survival in comorbidity of heart failure and type 2 diabetes does not exceed 25% [5, 6, 7]. The urgent task is to look for preventative measures of heart failure in patients with type 2 diabetes. It includes the improvement of glucose-lowering therapy, namely the use of drugs that do not only improve glycaemic control but also reduce the risk of cardiovascular diseases and heart failure. Recently, an arsenal of agents for the treatment of type 2 diabetes has been supplemented with sodium-glucose co-transporter 2 inhibitors. They are associated with a decrease in glucose reabsorption in the proximal tubules of the kidneys, provided that the glomerular filtration rate is maintained.

THE AIM

is to study the possibilities of increasing the efficiency of prevention of cardiovascular diseases and heart failure when using a new class of glucose-lowering agents – sodium-glucose co-transporter 2 inhibitors.

MATERIALS AND METHODS

The analysis of the existing clinical and experimental data on the effect of SGLT-2 inhibitors on the cardiovascular system, kidney status, risk factors for cardiovascular complications.

REVIEW AND DISCUSSION

The study of new pathogenetic mechanisms responsible for maintaining chronic hyperglycaemia has led to the creation of a promising class of glucose-lowering agents that reduce plasma glucose by inhibiting glucose reabsorption in the proximal tubules of kidneys without hypoglycaemia [8]. Due to its mechanism of action, SGLT-2 inhibitors are not only able to improve glycaemic control but also to provide cardioprotective and nephroprotective effects in patients with type 2 diabetes and high cardiovascular risk.

The EMPA-REG OUTCOME study evaluated the effects of oral intake of 10 and 25 mg of empagliflosin on cardiovascular morbidity and mortality in patients with type 2 diabetes and high cardiovascular risk. The results have shown a 14% reduction in the risk of serious side cardiovascular events (severe cardiovascular event; non-fatal myocardial infarction; non-fatal stroke and mortality from cardiovascular disease), a decreased risk of cardiovascular mortality by 38% and overall mortality by 32%, hospitalization frequency with heart failure by 35% on receiving empagliflozin compared with placebo [9]. A low risk of acute heart failure was observed in patients receiving empagliflozin with a history and without a history of heart failure [10]. The favorable effect of empagliflozin on reducing deaths and hospitalizations due to heart failure was common in patients throughout the observation period and after discontinuation of the drug (up to 3.1 years). Both doses of empagliflozin (10 mg and 25 mg) had similar effects on cardiovascular outcomes, so this effect was not dose-dependent. Besides the effects on cardiovascular risk, based on additional data from EMPA REG-Outcome, it has been shown that the addition of empagliflozin to the standard therapy significantly slowed the development and progression of kidney damage in patients both without albuminuria and with microalbuminuria and had the greatest effect on patients with the most severe course of nephropathy (existing macroalbuminuria) [11].

Prior to the publication of the results of the EMPA-REG study, the ability to improve the prognosis of patients with heart failure had not been proven for any of the glucose-lowering agents, moreover, some of these agents can impair it [12].

Positive results of SGLT-2 inhibitors were also obtained in another clinical trial program, CANVAS, which examined the safety and efficacy of canagliflozin in patients with type 2 diabetes who had a history of cardiovascular events (65%) or with a high cardiovascular risk (35%). Adding canagliflozin to the standard therapy not only reduced blood pressure and body weight, but also reduced the rate of hospitalization for heart failure by 33%. The drug also showed a nephroprotective effect due to its ability to slow albuminuria progression by 27% and increase albuminuria regression by 70%. The level of an estimated glomerular filtration rate was stable in the main canagliflozin group whereas it decreased in placebo patients [13].

A large international SVD-REAL study evaluating the data of more than 300,000 diabetic patients (87% of patients did not have cardiovascular disease at the beginning of the study) has demonstrated that SGLT-2 inhibitors compared to other diabetes medications are associated with a 39% relative reduction in the risk of hospitalization for heart failure and overall mortality by 51% [14]. In another CVD-REAL2 international study, the use of SGLT-2 inhibitors was associated with a 49% reduction in the risk of death and a 36% reduction in the risk of hospitalization for heart failure [15]. It should be noted that the vast majority of patients in this study did not have a diagnosis of cardiovascular disease. Therefore, the findings in the CVD-REAL and CVD-REAL2 studies regarding the cardiovascular benefits of SGLT-2 inhibitors can be extrapolated to a broader population of patients with diabetes.

It is not yet fully understood what underlies the cardioprotective action of SGLT-2 inhibitors. These mechanisms are most likely not associated with glycaemia reduction. A number of additional effects of SGLT-2 inhibitors (the so-called pleiotropic non-hypoglycaemic effects) may be viewed on the example of empagliflozin. It is broadly accepted that patients with diabetes are known to be characterized by an excess of Na +, due to its increased reabsorption in the kidneys by hyperglycaemia, hyperinsulinemia, activation of the renin-angiotensin-aldosterone system, etc. [16].

The delay of Na + and water plays an important role in increasing the preload and afterload of the heart, leading to the development of peripheral oedema and stagnation of blood in the lungs, and eventually to hospitalization. In this case, the excess of Na + is distributed not only in extracellular space, but also inside cells. The excessive content of Na + inside cardiomyocytes increases the risk of arrhythmia in the experiment and may lead to impaired myocardial function, in particular through impaired mitochondrial function [17].

Empagliflozin has the properties of an osmotic diuretic and enhances natriuresis. By reducing the volume of blood plasma, it helps to achieve euvolaemic state and lower overload on the ventricles of the heart and, consequently, reduce the volume overload of the heart, which can play a role in reducing the risk of arrhythmias and arrhythmic death. Reduction of peripheral vascular resistance and blood pressure can reduce cardiac afterload, improve coronary blood flow and myocardial contractility. Among the important additional advantages there are the lack of activation of the sympathetic nervous system and the absence of changes in potassium levels, since the occurrence of hyperkalaemia leads to a decrease in the positive effect of diuretic therapy on the frequency of cardiovascular events [18]. This may attribute empagliflozin to diuretics with unique properties, and it is likely that SGLT-2 inhibitors will be more in demand, cardiology included, than conventional diuretic therapy after further large-scale studies.

Several studies have shown that SGLT-2 inhibitors cause a decrease in systolic blood pressure in the range of 3-5 mm Hg and diastolic blood pressure – 2-3 mm Hg, reduce the pulse pressure and the average blood pressure [19]. In particular, in the EMPA-REG Outcome study, the reduction in systolic blood pressure after empagliflozin was 4 mm Hg, while diastolic blood pressure was 2 mm Hg, which was not accompanied by an increase in heart rate. This leads to the conclusion that there is no compensatory reflex activation of the sympathetic nervous system and suggests the effect of SGLT-2 inhibition on the reduction of the stiffness of the arterial vessel wall. Thus, the use of empagliflozin in young patients with an uncomplicated course of type 1 diabetes [20] led to a decrease in systolic blood pressure (an average of 2.7 mm Hg). It was also noted that the drug reduced the speed of the pulsatile wave and of the radial artery.

Therefore, reducing the incidence of cardiovascular complications and mortality in patients with type 2 diabetes compared with other diabetes-reducing agents or placebo may primarily be related to the hemodynamic effects of SGLT-2 inhibitors [21, 22, 23], which reduce preload and afterload, vascular stiffness, which improves left ventricular function and leads to a decrease in myocardial oxygen consumption.

In people with type 2 diabetes, SGLT-2 inhibitors, in addition to the antihypertensive effect, have caused dose-dependent weight loss as a result of the osmotic diuretic effect and loss calories for glucosuria [24, 25, 26] – excretion of 50-80 g of glucose a day stands for losing 200-300 kcal. In addition, another important aspect is a lower level of insulin, which is for its anabolic effects, as well as an increase in the oxidative metabolism of adipose tissue [27]. This results in gradual (usually within a few months) reduction of the body mass by 2-3 kg, with the further stabilization of body weight after 3-6 months [28]. Investigating the results of EMPA-REG Outcome in the course of empagliflozin treatment, the lowering of an average body weight is pointed out by 2 kg, resulting in a decrease in waist by 2 cm. The influence of SGLT-2 inhibitors on visceral fat is of a special interest. The latter is associated with a higher degree of probability of type 2 diabetes development, cardio vascular complications and death [29].

Among the beneficial extraglicaemic effects of SGLT-2 inhibitors there is a glucose-dependent decrease in urate reabsorption [30]. SGLT-2 inhibitors increase the excretion of uric acid and reduce its plasma concentration by 10-15%, which within the EMPA-REG OUTCOME is 24 μmol /l. For a long time, hyperuricemia was not only considered as a component of the metabolic syndrome, but was also associated with the decrease in cardiovascular diseases [31]. The accumulated data, both in humans and in experimental models, illustrate that the increased plasma level of uric acid may lead to arterial hypertension, endothelial vascular dysfunction, congestive heart failure, and kidney dysfunction [32].

The decrease in uric acid levels can hardly explain the rapid improvement in cardiovascular outcomes demonstrated by empagliflozin compared to placebo. Nevertheless, this effect may play a certain role in reducing cardiovascular mortality at a later period of drug administration and slow down the progression of diabetic nephropathy.

Empagliflozin does not increase the incidence of hypoglycaemia regardless of baseline glycated hemoglobin (HbA1c) concentration, which gives an opportunity to improve cardiovascular prognosis without increasing the risk of hypoglycaemia. The second benefit is that the risk of hypoglycaemia does not increase depending on the basic therapy. It should be emphasized that the mechanism of action of SGLT-2 inhibitors is insulin-independent. Therefore, SGLT-2 inhibitors can be used at any stage of type 2 diabetes, in particular in the depletion of beta-cellular apparatus of the pancreas. Thus, they can be combined with other anti-hyperglycaemic drugs, since their mechanisms are different from the mechanisms of action of other currently available classes of drugs [33]. SGLT-2 inhibitors are the only class of glucose-lowering agents, other than metformin, that are, without any limitation, compatible with basal insulin. The addition of SGLT-2 inhibitors to the therapy regimen helps to improve glycaemic control with a lower insulin dose.

Numerous data have convincingly shown that chronic kidney disease resulting from diabetic nephropathy is an important and independent risk factor for cardiovascular pathology. Population studies have shown that a combination of the chronic kidney disease and type 2 diabetes in a patient significantly increases the incidence of cardiovascular complications. However, albuminuria has also been identified as a risk factor for death from a cardiovascular disease. The analysis of a numerous randomized placebo-controlled clinical trials, including EMPA-REG OUTCOME, CANVAS, has shown that SGLT-2 inhibition can cause both cardiovascular and renoprotective effects [34, 35, 36, 37].

SGLT-2 inhibitors have been proven to prevent impaired glomerular filtration and reduce the degree of albuminuria in patients with diabetes-related kidney disease. In the Renal branch of the EMPA-REG OUTCOME study, there was a significantly lower risk of progression to macroalbuminuria or other clinically relevant renal outcomes, such as doubling of serum creatinine levels and initiation of renal replacement therapy in patients from the empagliflozin group in comparison to the placebo one. Thus, the obtained results allow us to confidently attribute empagliflozin to drugs with a nephroprotective action. The mechanism of nephroprotective action of empagliflozin, apparently, is not only due to the decrease in glycaemia, but also due to the non-glycaemic effects in the form of weight loss, decrease in blood pressure and stiffness of the arterial wall, correction of intrarenal hemodynamics, increase of natriuresis. Nephroprotection can be associated with the direct effect of empagliflozin on inflammatory processes, since inflammation, fibrosis and oxidative stress are closely related to intracellular hypertension [38]. The results of in vitro and in vivo studies suggest that inhibition of SGLT-2 slows the activity of these processes [39]. However, in studies with empagliflozin, inflammatory markers have not been evaluated, although there is some evidence that C-reactive protein reduced dapagliflozin [40]. This will possibly be the subject of the further study. The results of studies on the prospects of using SGLT-2 inhibitors for the treatment of heart failure in individuals without diabetes will also be of great interest.

CONCLUSIONS

1. When choosing glucose-lowering agents in patients with diabetes, it is necessary to consider their impact on the risk of development and the course of heart failure.
2. SGLT-2 inhibitors should be considered as a preferred method of treatment for diabetes in patients with heart failure or with a risk of heart failure, which meets the recent recommendations of the European and American Diabetes Association.

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CASE REPORT
OPIS PRZYPADKU

DIAGNOSTIC PROBLEMS ACCOMPANYING BRANHYOGENIC CANCER – A CLINICAL CASE

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ABSTRACT

Tumours and tumorous lesions of head and neck account for 10% of all oncological pathologies. Branhyogenic cancer is found in 4.5% of patients with lateral cysts in the neck. The article highlights the results of research the clinical case of branhyogenic cancer, provide its clinical and morphological analysis. The aim of our work was to study the clinical case of bronchial cancer, providing clinical and pathomorphological analysis. Examination and treatment was conducted in accordance with the clinical protocol using the diagnostic criteria necessary for management of patients diagnosed with tumours and tumorous lesions in a particular clinical case. We applied ultrasound examination of the locus, angiography of head and neck vessels with tomohexol and with 3D reconstruction, histological examination of surgical specimens (macroscopy and microscopy). On the basis of clinical investigaton, ultrasound examination, angiography clinical diagnosis was formulated – lateral cyst on the left side of the neck. A radical surgical removal of the mass was conducted. Histopathological conclusion: there is a proliferation of cystic transitional cell epithelium with the locus of invasive squamous cell carcinoma in the cystic wall that suggests malignant transformation of bronchogenic cyst. Final diagnosis: branhyogenic cancer.

Thorough examination and analysis of a clinical case demonstrates that the development of branhyogenic cancer, is histo-genetically associated with lateral cysts in the neck. Complexity of diagnosing and high percentage of malignancy induces to more early discovery and removal of lateral cysts in the neck .

KEY WORDS: branhyogenic cancer, lateral cysts in the neck

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INTRODUCTION

Prevalence rate of oncologic pathology grows increasingly. In the structure of morbidity of malignant tumours the neck lesions do not constitute a very high percentage – tumours and tumorous lesions of head and neck account for not more than 10% of all oncological pathologies [1]. Branhyogenic cancer (BC) is found in 4.5% of patients with lateral cysts in the neck (LCN) [2]. The incidence of LCN and BC in men and women is equal. BC is more often diagnosed in people aged over 50 years, LCN is diagnosed in between the ages of 20 to 50 years [3, 4]. BC, defined as squamous cell carcinoma, is associated with abnormalities of gill slits and was first described by Folkman in 1882 [5]. Differential diagnostics of BC and LCN quite often is a complicated task. In the early stages of these diseases there are no complaints, later on – carcinomatous mass appears on the lateral area of the neck which is increasing gradually. Duration of disease (from the moment of discovering a tumour until surgery) is different: up to 6 months – 71.8% of patients, from 6 months to 1 year – 15.4%, more than 1 year – 12.8% [6]. BC quickly grows in the surrounding tissues, its mobility becomes limited, and precision of contours becomes lost. Palpation is painful, the surface is nodular. Metastases to the lymph nodes are rarely observed.

LCN constitute nearly 1.4% -2.2% of all dental diseases, 5% of all lesions of the face and neck and 25% of all soft tissue cysts of maxillofacial area [7, 8]. Pathogenesis of LCN has been studied by the specialists for the past 2 centuries and today it is interpreted contradictorily. Scientists put forward bronchogenic, "thymic" limphoepithelial, genetic theories and the possibility of LCN occurrence under the influence of various teratogenic factors during the first weeks of pregnancy [9, 10, 11].

BC and LCN have typical localization: in the upper, just at the angle of the lower jaw (33%) or middle third of lateral area of the neck (67%) on the inner edge of sternocleidomastoid muscle, directly on the neurovascular bundle of the neck at the level of bifurcation of the common carotid artery, sometimes partly extending under the muscle. Tumours are adjacent to the internal jugular vein [12, 13].

Diagnostics of BC and LCN neck cysts cause some difficulties for the physicians. In 9-11% of cases the diagnosis of LCN, with which patient is sent to the hospital does not coincide with the clinical or postoperative diagnosis [14]. Such a high percentage of wrong diagnoses can be explained by scant clinical picture of these tumours and the lack of information obtained during the analysis of diagnostic methods [15, 16, 17].

Diagnostic puncture biopsy of tumours conducted before surgery is recognized by most authors as ineffective due to possible contamination of surgical field's tissues, in addition, it should be noted that conducting puncture and/or incisional biopsy in this area may be associated with the risk of bleeding [18]. In case of LCN the precision of ultrasound examination constitutes 45-50% [19]. There are a lot of diagnostic methods for tumours, but the gold

standard in oncology is considered to be histological study of the obtained material, since only this method enables to clearly identify a tumour. Some authors argue that the diagnosis of BC bears a theoretical and hypothetical character. Thus, the diagnosis itself remains controversial up to the present day [20, 21].

Bronchogenic cysts which were not diagnosed in time, prone to malignancy. This determines the relevance of early diagnosis and treatment of patients with tumours of the neck. A clinical case with tumour in lateral neck area is described in this study.

THE AIM

To study the clinical case of branhyogenic cancer, provide its clinical and patomorphological analysis.

CLINICAL CASE

The patient P., 38 years old, hospitalized to the Maxillofacial Surgery Department of Clinical Hospital of Emergency Medical Care in Lviv with complaints on the tumour in the submandibular and lateral neck areas on the left side. Provisional diagnosis: chronic lymphadenitis of submandibular and lateral neck areas. The diagnostics of the disease included clinical data, ultrasound examination, angiography of head and neck vessels with tomohexol and with 3D reconstruction, histological examination of surgical specimens (macroscopy and microscopy). Microscope Nikon E200 with photocamera Nikon 5000 of ×80, ×200 and ×400 zoom was applied for taking micro photos. The patient underwent radical surgery.

According to the words of the patient, 1.5 years ago painless swelling in the left side of the neck appeared increasing gradually. Diagnostics was based on clinical data and the results of additional investigation methods. General physical examinations and plain chest radiography were done without presence of abnormalities. The skin over the tumour was not changed in colour, mobile. The tumour was of tight elastic consistency, painless, limited, rounded, with a smooth surface. The formation was painless, undulated during palpation, pulsation of the carotid artery was not transmitted. When turning head in the opposite direction bulging of sternocleidomastoid muscle became well contoured at the front (Fig. 1).

The tumour did not cause compression of organs located around it: there was no breathing difficulty, no violation of swallowing, no dry cough, dystonia. Lymph nodes of the skull, facial, genian, submandibular, neck lymph nodes were non-palpable. Data of ultrasound examination: on the left side of the lateral neck area there were liquid heterogeneous formations with d from 30 mm to 20 mm (3 pieces) with the capsule, limited, with no blood circulation. No enlargement of lymph nodes. Description of angiography examination: the thyroid gland was located typically, of normal shape and size. Neck lymph nodes were not enlarged. At the level of bifurcation of the external carotid artery on the left side two clearly contoured formations containing liquid with density of 27 HU, with clear outer contour and uneven inner contour, with accumulation of contrast medium in the form of the wall up to 25 mm was visualized. On the upper contour of tumour another tissue formation of homogeneous structure with signs of accumulation of contrast up to 17 mm was visualized. Formations were located under the front edge of sternocleidomastoid muscle. On their inside retromandibular vein and external carotid artery passed (Fig. 2, 3, 4).

Conclusion: cystic tumours may correspond to the cysts of the second gill arch (second bronchial cleft cysts). On the basis of the obtained data a clinical diagnosis was formulated – lateral cyst on the left side of the neck. Under endotracheal anaesthesia surgery on removal of tumour of the lateral area on the left side of the neck was conducted. During the surgery, special attention was paid to manipulations near the vascular-nervous bundle of the neck and radicality of intervention itself. After antiseptic preparation of the surgical field a cut on the front edge of sternocleidomastoid muscle, with the length of 10 cm, extending to the ear lobe, was made; skin, subcutaneous tissue, superficial muscle of the first and second neck fascia, neurovascular case were passed by, formation allocated in the membrane, with the size of about 8.0×4.0 cm containing liquid, that occupied submandibular area and lateral neck area was detached (Fig. 5, 6).

On the inside it was adherent to the membrane of external carotid artery, along it, retromandibular vein delegation was conducted and peripheral branches of the facial nerve were detached. Haemostasis. The wound was stitched with nylon 3-0 layer by layer, Vicryl sutures, drainage were put on the skin. Pressing aseptic bandage. Tumour in the capsule was sent to histopathological study. Macro and microscopy description: the researched surgical specimen is presented by hollow formation of pink-red colour 4×2.5 cm, with greenish, viscous content at the crosscut and tumorous formation with d = 1.5 cm of grey colour. Histopathological conclusion: there is a proliferation of cystic transitional cell epithelium with the locus of invasive squamous cell carcinoma in the cystic wall that suggests malignant transformation of bronchogenic cyst (Fig. 7, 8, 9). Final diagnosis: branhyogenic cancer.

CONCLUSIONS

LCN and BC have rather similar clinical and morphological picture, require reasonable complex examination and surgical treatment to prevent diagnostic errors and recidivation, and mandatory verification of a tumour. Occurrence of three cystic tumours in one patient is quite rare. The detailed study of clinical case proves that the development of BC is histo-genetically associated with LCN. A metaplasia of epithelium that covers the cyst with further malignancy occurred.

To sum up, we may state complexity of diagnosing and high percentage of malignancy induces to more early discovery and removal of LCN.

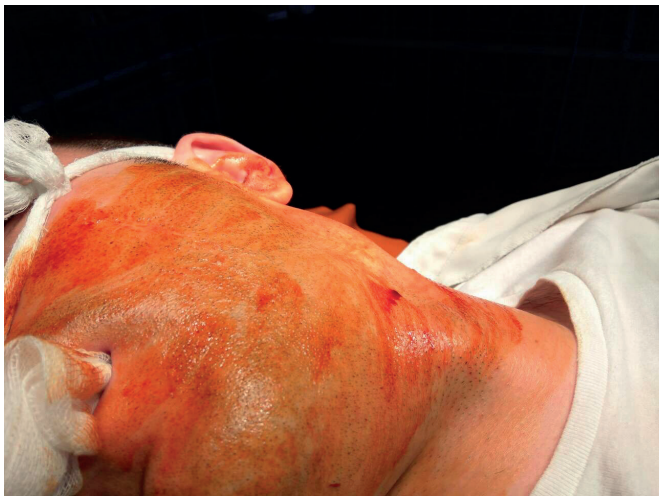

Fig 1. The general appearance of the patient suffering from the Branhyogenic cancer

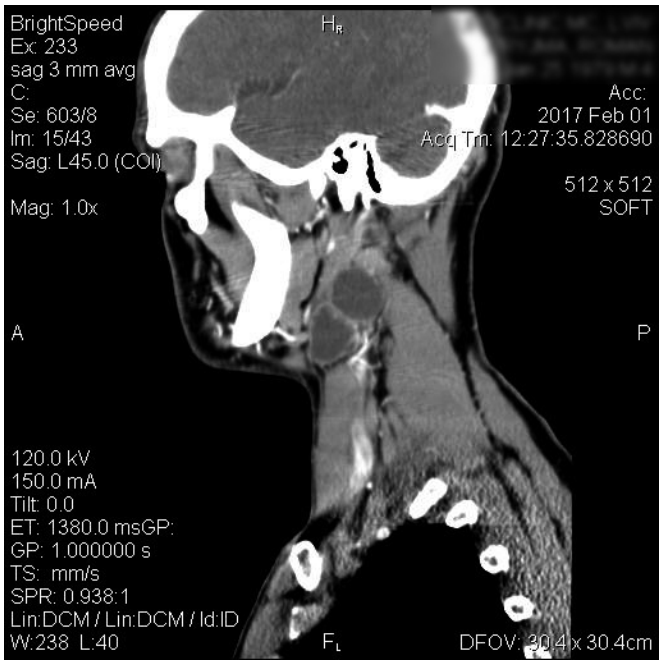

Fig 3. Plan angiography of head and neck vessels

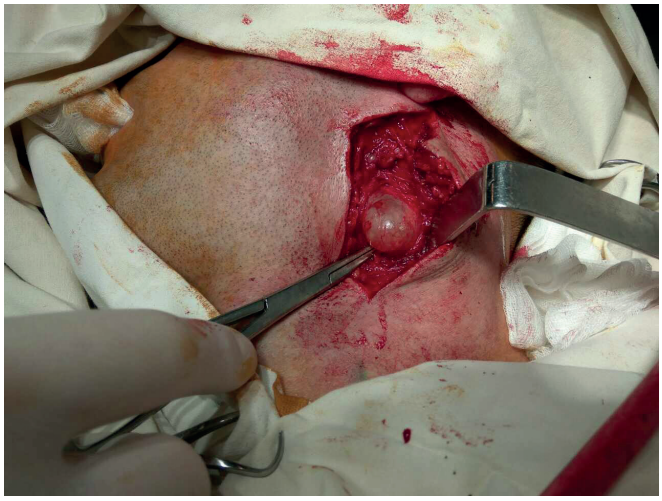

Fig 5. Intraoperative image of of the Branhyogenic cancer to surgical excision.

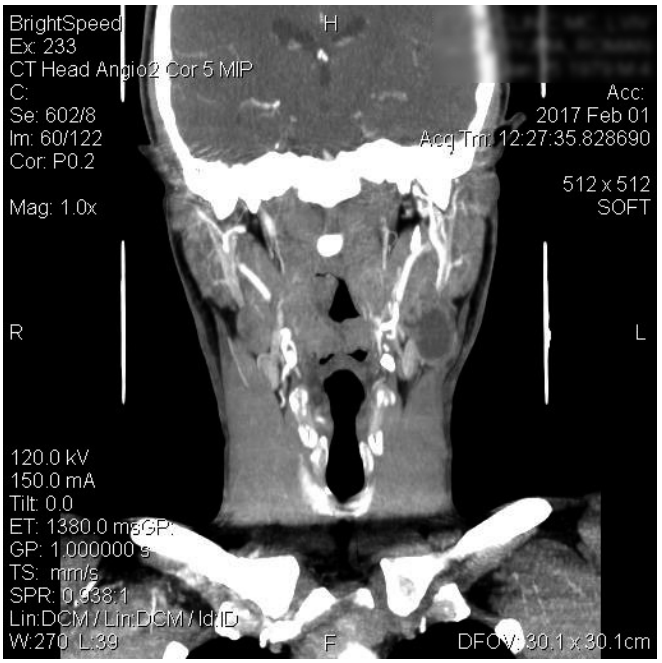

Fig 2. Plan angiography of head and neck vessels

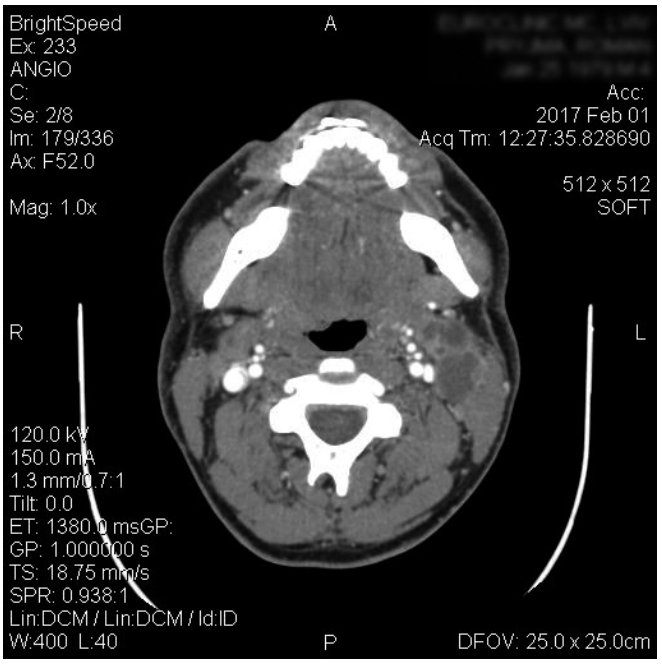

Fig 4. Plan angiography of head and neck vessels

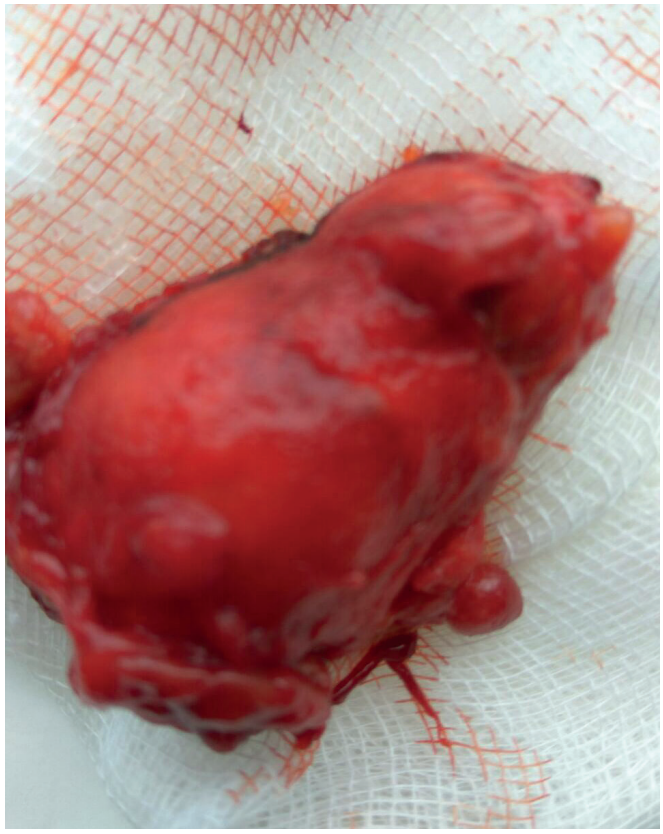

Fig 6. Branhyogenic cancer

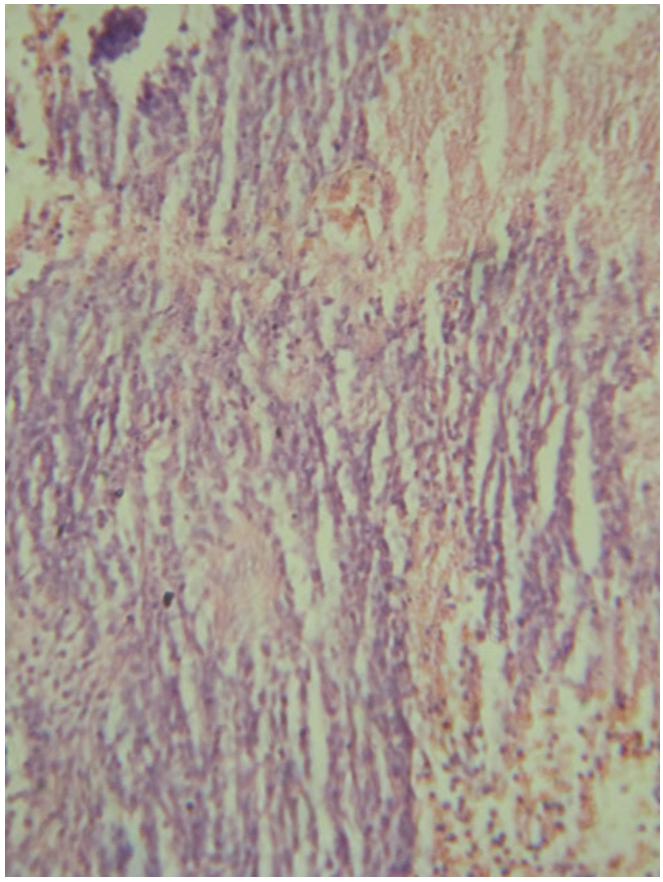

Fig 8. Branhyogenic cancer. Histological study № 3443–2/2017 (H&E, × 200)

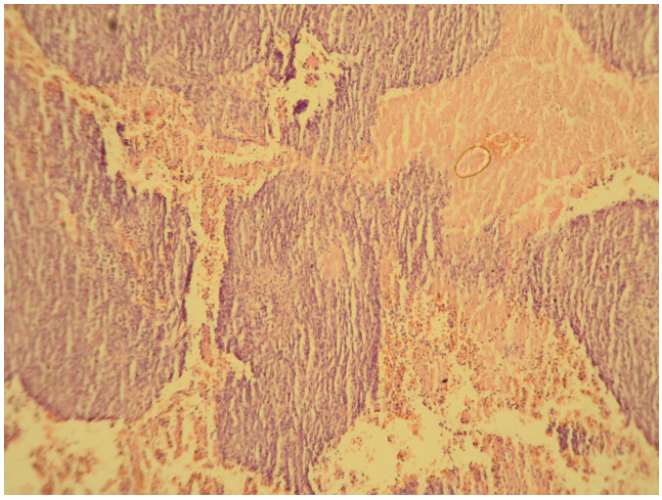

Fig 7. Branhyogenic cancer. Histological study № 3443 –2/2017 (H&E, × 80)

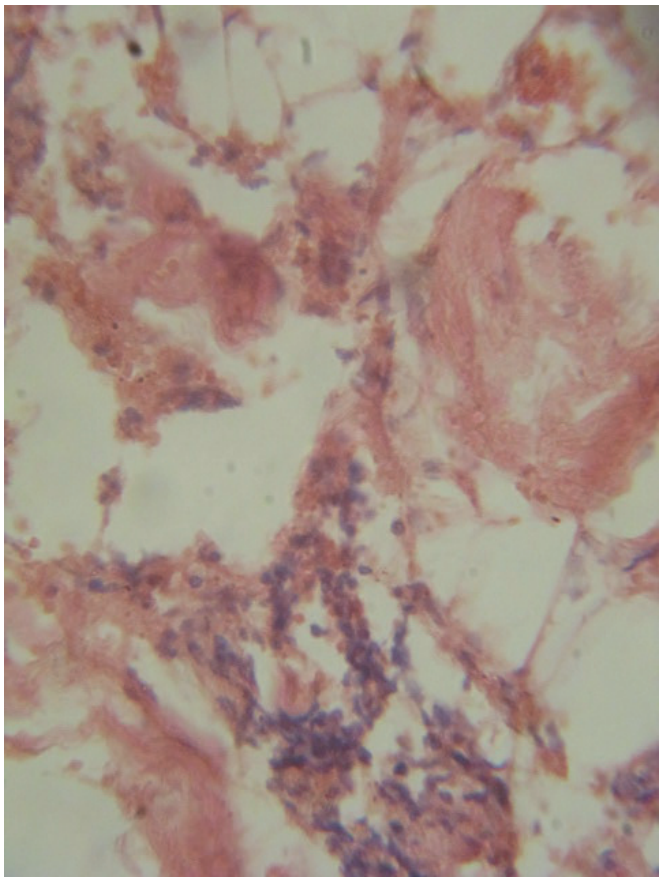

Fig 9. Branhyogenic cancer . Histological study № 3443 –2/2017 (H&E, × 400)

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