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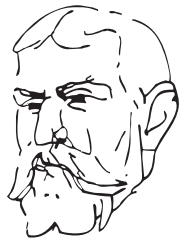

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REVIEW ARTICLES

ORIGINAL ARTICLE

EVALUATION OF DYNAMIC CHANGES IN THE MICROCIRCULATION OF THE MUCOSA IN THE ZONE OF DENTAL IMPLANTATION WITH IMMEDIATE INTRAOPERATIVE LOAD

DOI: 10.36740/WLek202309101

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ABSTRACT

The aim: Study of the dynamics of changes in the average values of the index of mucosal microcirculation after dental implantation with immediate intraoperative prosthetics**.**

Materials and methods: In clinical conditions, 55 patients aged from 29 to 60 years with a diagnosis of partial absence of teeth requiring orthopedic treatment using implants on the lower jaw were treated and examined. In the course of the latest achievements, the following methods were used: clinical protocol of immediate implantation with Solidum and Simplex implants of the «ART IMPLANT» system on the lower jaw by the one-stage implantation method, with immediate intraoperative loading and the manufacture of a temporary non-removable dental prosthesis, determination of the microcirculation index in dynamics using the laser Doppler method flowmetry, statistical analysis.

Results: The obtained results indicate a pronounced reaction of microcirculation up to the 3rd day after surgery, an increase in blood perfusion of the mucous membrane by 2.7 times while maintaining vasomotor activity, which indicates adaptation to the injury and immediate loading of the denture in the postoperative period. 3 months after dental surgery and immediate intraoperative prosthetics, all indicators of microcirculation approach the initial values before surgery.

Conclusions: With the help of laser Doppler flowmetry, the fact of a sharp restoration of microcirculation after dental implantation surgery with immediate intraoperative prosthetics is confirmed.

KEY WORDS: Laser Doppler flowmetry, immediate intraoperative prosthetics, immediate implantation, indicators of microcirculation, implants of the «ART IMPLANT» system, osseointegration.

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INTRODUCTION

As a result of monitoring and analysis of clinical and experimental studies on the success of dental implantation in the early and distant periods of observation, scientists managed to substantiate the feasibility of introducing into clinical practice not only classical, but also modified protocols of implantation with different terms of subsequent loading [1,2]. At the 6th ITI (International Team of Implantologists) Consensus Conference, held in Amsterdam in April 2018, a group of scientists led by German Gallucci developed a unified classification of dental implant installation and loading protocols for all practically possible clinical situations. Based on the analysis of the publications selected for monitoring that met the evidence criteria, the implant survival rates were calculated with the determination of success and predictability of each protocol [3,4]. Today, there is a trend towards shortening the terms of subsequent orthopedic rehabilitation. Immediate implantation and immediate intraoperative functional masticatory load allow to reduce the duration of treatment and the volume of surgical intervention and to obtain a high functional and aesthetic result [4]. Today, there are discussions about the features of the formation and level of reduction of peri-implant bone tissue in various conditions, in particular, under load and micromobility [5]. It has been established that around loaded implants there is an increase in blood flow and vasomotor activity of the microcirculatory bed of the supporting tissues, an increase in the volume of bone tissue and an increase in torque, which is an optimal prediction of the acceleration of the rate of osseointegration [6]. The modern systematization

of views on the differentiation of the results of immediate loading and immediate implantation and their interpretation from the point of view of the modern understanding of the mechanisms of bone remodeling expands the opportunities for discussion and argumentation of the prognosis of various dental implantation protocols taking into account the initial conditions of the clinical situation [7,8]. There are a large number of methods and devices that allow you to evaluate various characteristics of the movement and distribution of blood in the body, starting from the simplest: visual observation, listening, palpation, etc. In the 17th century, Marcello Malpighi invented the liquid plethysmograph. From that moment, the era of instrumental blood circulation research began [9,10]. In domestic implantology, standard examination methods do not allow detecting disorders that occur in the mucous membrane area during dental implantation. Therefore, the use of highly informative methods of assessing hemodynamics and metabolism reveal hidden mechanisms of disease development. To date, the study of the state of microcirculation is carried out using various methods, for example, such as laser diagnostics. With the development of science and technology, new opportunities for blood flow research appeared, in particular with the help of laser Doppler flowmetry [11]. The use of laser Doppler flowmetry (LDF) to analyze capillary hemodynamics in real time made it possible to determine microcirculation changes during dental implantation, both two-stage and with immediate loading. The LDF method is based on the use of low-power helium-neon laser radiation (Λ = 632.8 nm), which penetrates well into the surface layers of tissues. When radiation is reflected from moving objects (which are erythrocytes in microvessels), there is a change in the frequency of the signal (Doppler effect). Determination of the intensity of microcirculation in tissues is based on this effect [12-16]. An early local sign of pathological disorders is vasoconstriction of arterioles, stagnation in venules and a decrease in blood flow in nutritional capillaries. At the same time, the rhythmic changes of hemodynamics in the microcirculatory channel are disturbed. Successes in the clinical application of the results of microcirculatory studies are associated with the use of capillaroscopy, assessment of microblood flow by laser Doppler flowmetry, and others. The clinical importance of studying microcirculation is determined by the possibility of early detection of tissue viability disorders [17]. Understanding the negative and early terms of implant loading under the influence of functional occlusive loading in conditions with expert analysis of hemodynamic changes in the microcirculatory channel around implants is limited in fundamental research. Also, there is not enough convincing experimental data on the dynamics of changes in the average values of the microcirculation index (IM) after dental implant surgery and immediate intraoperative prosthetics.

THE AIM

The aim of the study was to study of the dynamics of changes in the average values of the index of mucosal microcirculation after dental implantation with immediate intraoperative prosthetics.

MATERIALS AND METHODS

The clinical study was conducted on the basis of the Department of Post-Graduate Education Dentistry of the Uzhhorod National University, the dental clinic «Art dentistry» (Zaporizhia, Ukraine), the university dental clinic of Debrecen University (Debrecen, Hungary). The study was carried out taking into account 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 under the Presidium of the National Academy of Sciences of Ukraine (2002) and the positive opinion of the bioethics commission of the Uzhhorod National University .

In clinical conditions, 55 patients (25 male and 30 female patients), aged from 29 to 60 years with a diagnosis of partial absence of teeth (10 - 08.1), requiring orthopedic treatment using implants on the lower jaw, were treated and examined. Prosthetics were performed with fixed orthopedic structures. In order to replace the defect, 65 Solidum and Simplex implants of the «ART IMPLANT» system were installed on the lower jaw by the method of one-stage implantation, with immediate intraoperative loading and the manufacture of a temporary non-removable dental prosthesis. Prosthodontics were performed on 65 implants, 65 temporary plastic crowns were made, and then 65 ceramic or metal-ceramic crowns (Table I). All patients were fitted with dental implants in the defect area of 1-2 teeth on the lower jaw with immediate loading, using intraoperative prosthetics.

The inclusion criteria were - partial loss of teeth, the extent of the defect of 1-2 teeth on the lower jaw (Fig. 1). Satisfactory or good level of oral hygiene, healthy mucous membrane of the oral cavity, sufficient volume of the jaw bone and its satisfactory density, sufficient space for installing a dental prosthesis, absence of endocrine diseases.

Table I. Distribution of used implants

Implantation system	Number of implants	Number of crowns	Dentition defect	
			1 tooth	2 teeth
Solidum system «ART IMPLANT»	32	32	8	7
Simplex system «ART IMPLANT»	33	33	31	4
Together	65	65	39	11

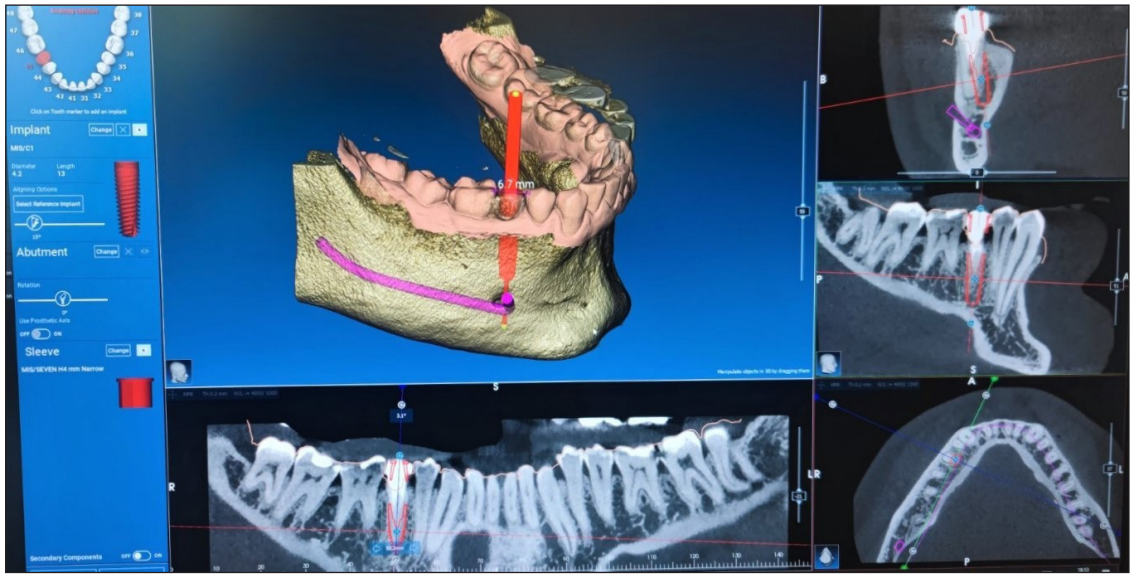

Fig. 1. Clinical situation before implantation.

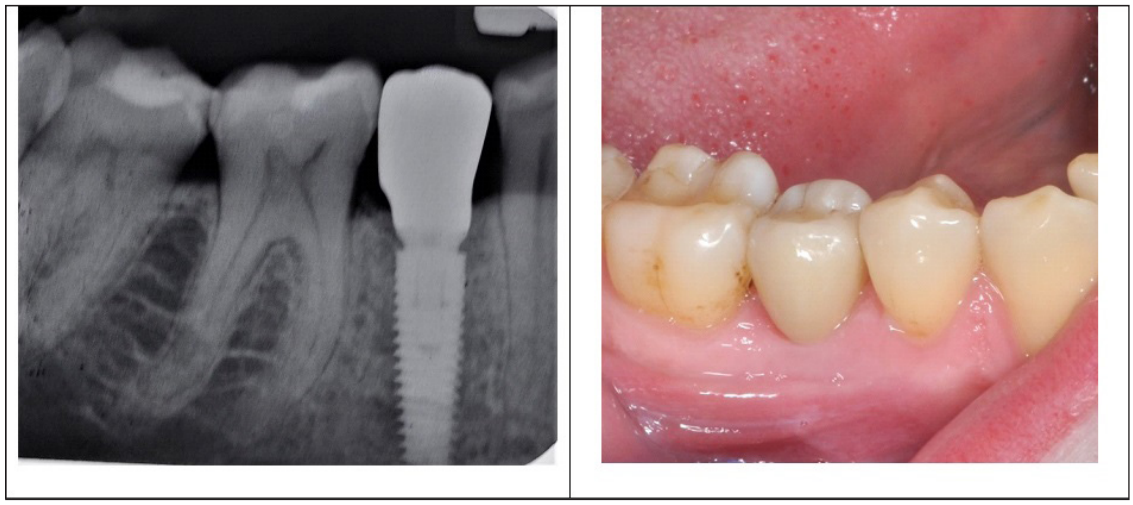

Fig. 2. Clinical situation after implantation

The exclusion criteria were – lack of positive motivation for treatment with the use of dental implants, age of the examinee over 65 years, low level of oral hygiene, alcohol abuse and tobacco smoking, chronic generalized periodontitis of medium and severe degrees, significant atrophy of the alveolar part in the area of the dentition defect of the lower jaw , insufficient density of the jaw bone, osteoporosis, insufficient prosthetic space, presence of endocrine diseases, immunodeficiency, blood diseases, radiation and chemotherapy, systemic corticosteroid therapy, chronic somatic diseases in the stage of decompensation. Planning and treatment of patients was carried out according to the following algorithm: cone-beam computed tomography was performed to obtain files in dicom format for further planning of dental implant installation (Fig. 1). In the future, after intraoral scanning with an optical scanner to obtain a file in STL format, dicom and STL format files were combined in the 3Diagnosis computer program to compile a complete clinical picture, which includes all anatomical formations of the selected area, a

virtual placement of the implant and its superstructure was carried out . A virtual model of the surgical template is created using the received data about the state of the implant in the PlastyCAD software. The template was printed using a 3D printer. Patients underwent immediate implantation with passive occlusal loading according to traditional one-stage surgical protocols (Schwartz-Arad D. et al., 2007) under Sol local anesthesia. Articaini 4% with a vasoconstrictor 1: 100,000.

After the surgical stage of dental implantation, an optical transfer was installed in the joint of the implant. After obtaining an optical impression, the transfer was temporarily changed to a gingival former (Fig. 2). After the laboratory production of the temporary superstructure using a RolandDWX-51D milling machine, it was installed in the joint of the implant with a force of 10-15 N.

To evaluate the results of microcirculation research using the laser method Doppler flowmetry (LDF) in patients with partial adentia, the condition of the frontal and lateral areas of the lower jaw was studied using system moor VMS LDF2 (785 ± 10 nm, maximum power 2.5mW) (Moor Instruments Ltd. Millwey Axminster Devon EX13 5HU, UK) (Fig. 1) and needle probes as per the manufacturer's instructions, and were processed by the use of specialized software (moor VMS-PC, V 3.1 for Vascular Monitor System).

The results of laboratory and clinical studies were processed by methods of variational statistics with determination of the mean value, its errors, Student's t-test for multiple comparisons, using Excel (MS Office 2010, Microsoft, USA) and STATISTICA 6.0 (StatSoft, USA). Differences in indicators at the level of significance p <0.05 were considered statistically significant.

RESULTS

According to the data of the patients before the dental implantation operation, the integrated indicator of microcirculation (IM), which reflects the level of blood flow in the mucous membrane, was on average 8.50 ± 0.43 points. (Fig. 3). At the same time, the temporal variability of blood perfusion σ, which characterizes fluctuations in the flow of erythrocytes in the microcirculatory channel, was 0.90 ± 0.04 p.o. (Fig. 4). Vasomotor activity of microvessels (Kv) was equal to 10.59 ± 0.48% (Fig. 5). After dental implant surgery with immediate intraoperative prosthetics, after 3 days IM increased sharply by 2.7 times (P < 0.001) and the fluctuation of erythrocyte flow (σ) also increased by 3.1 times (P < 0.001) (Fig. 3, 4).

However, vasomotor activity (Kv) increased by 7.06%, which indicates compensatory reactions of the microcirculation system in the mucosa (Fig. 5). 10 days after the operation, the level of blood flow in the microcirculatory channel of the PM began to decrease, but it significantly exceeded the initial values by 125.88% (p <0.001). Fluctuation of the flow of erythrocytes (σ) in the microcirculatory channel also had a tendency to decrease in amplitude, while its values exceeded those before surgery by 123.33% (p <0.001). Kv at the same time remained approximately at the same level

Table II. Indicators of vascular tone in the microcirculatory channel of the mucous membrane in the area of installation of dental implants with immediate load according to the wavelet analysis of LDF- grams.

	Before the operation	3 days after surgery	10 days after surgery	After the operation in 1 month	3 months after the operation
			NT		
n	25	23	23	21	19
M	3,57	4,07	2,50	2,88	4,71
m	± 0,16	± 0,18	± 0,11	± 0,13	± 0,21
p <		0,05	0,001	0,05	0,05
			MT		
n	25	23	23	21	19
M	2,22	2,19	4,41	2,46	2,75
m	± 0,10	± 0,10	± 0,20	± 0,11	± 0,12
p <			0,001		0,05
			SHI		
n	25	23	23	21	19
M	0,62	0,54	1,77	0,85	0,59
m	± 0,03	± 0,02	± 0,08	± 0,04	± 0,03
p <		0,05	0,001	0,05	

Notes: p - significant differences after surgery

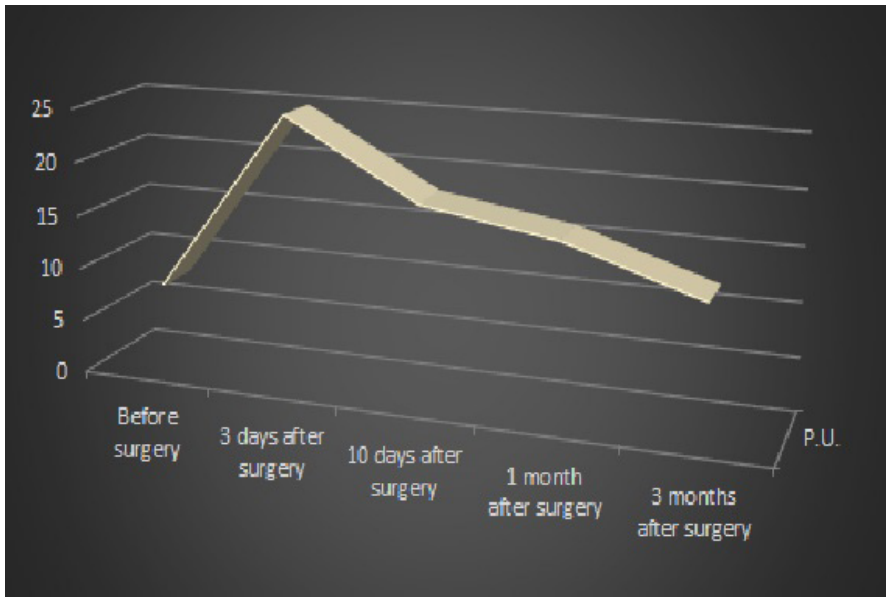

Fig. 3. Dynamics of changes in the average values of the microcirculation (IM) index after dental implant surgery and immediate intraoperative prosthetics

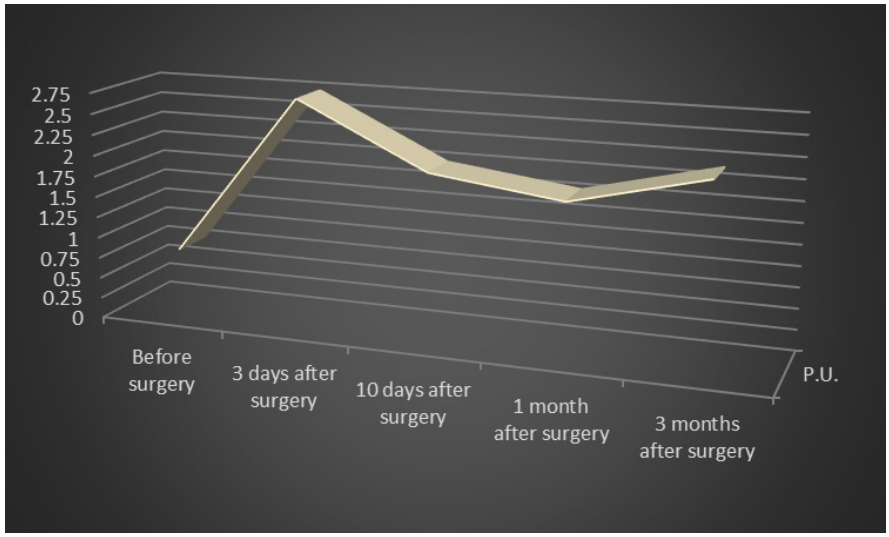

Fig. 4. Dynamics of changes in the average values of erythrocyte flow fluctuations (σ) after dental implant surgery and immediate intraoperative prosthetics

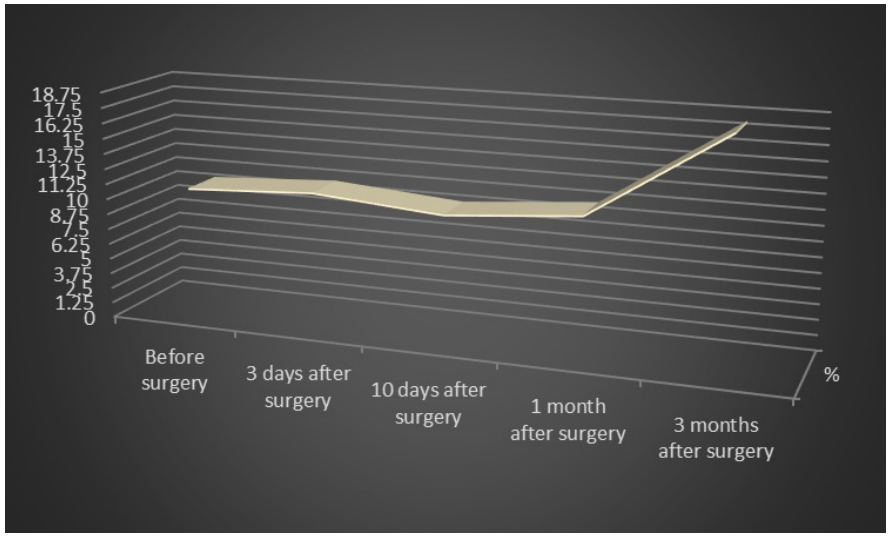

Fig. 5. Dynamics of changes in the average values of the coefficient of variation (Kv) after dental implantation surgery and immediate intraoperative prosthetics

(Fig. 3, 4, 5). After 1 month, a decrease in IM and σ was recorded to 16.90 ± 0.85 perf. units. and 1.90 ± 0.09 perf. units. in accordance. However, their values significantly exceeded the initial level by 1.99 and 2.11 times ($p < 0.001$). In the remote period, 3 months after the operation, the blood flow level (IM) in the mucous membrane was on average 8.50 ± 0.43 perf. units, which is 34.12% higher than the initial level before the

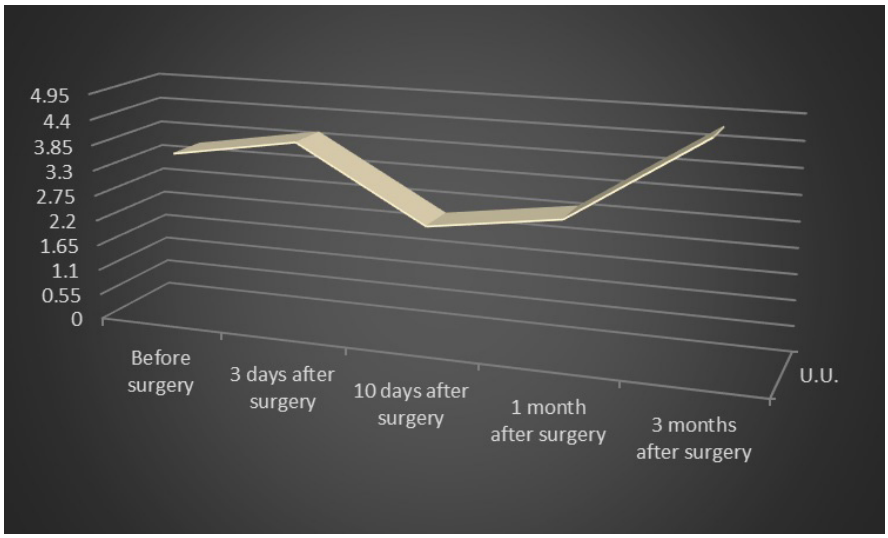

Fig. 6. Dynamics of changes in neurogenic tone (NT) after dental implant surgery and immediate intraoperative prosthetics according to wavelet analysis of LDF-grams

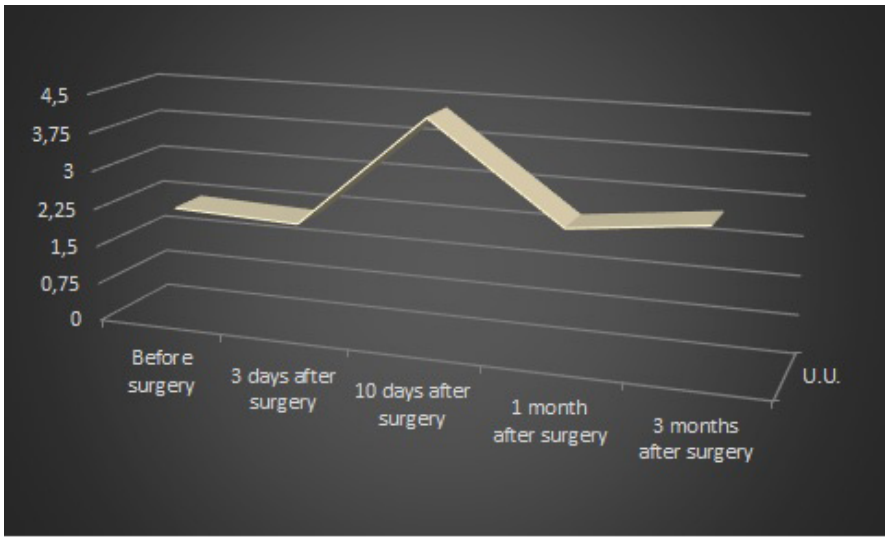

Fig. 7. Dynamics of changes in myogenic tone (MT) after dental implant surgery and immediate intraoperative prosthetics according to wavelet analysis of LDF-grams

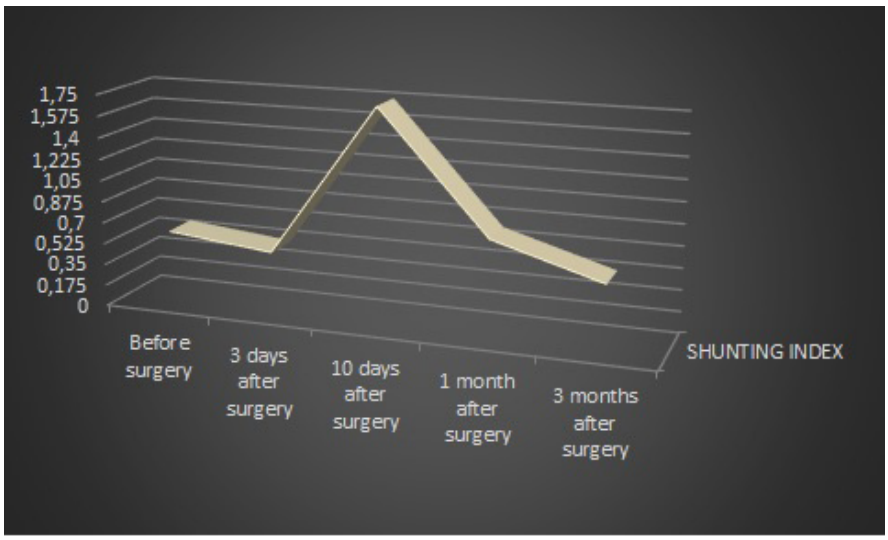

Fig. 8. The dynamic of changes in the shunt index (SHI) after dental implant surgery and immediate intraoperative prosthetics according to the wavelet analysis data of LDF – gram

operation ($p < 0.05$). At the same time, the fluctuation of the flow of erythrocytes (σ) in the microcirculatory channel was 2.10 ± 0.09 perf. unit increased by 133.33% ($p < 0.001$), which may indicate the improvement of the trophic function in the mucous membrane, which is an indicator of the restoration of the entire jaw bone complex. The analysis of these indicators, reflecting the vasomotor activity (Kv) in the microcirculatory channel, revealed an increase in its values by 73.98% compared to the preoperative values ($p < 0.001$) (Fig. 5).

The results of wavelet analysis of LDF - grams, presented in Table II, revealed an increase in the

neurogenic tone (NT) of precapillary vessels in the microcirculatory channel of the mucous membrane of the jaws 3 days after dental implantation with immediate loading by 13.93% ($p < 0.05$), and after 10 days - a decrease by 30.04% ($p < 0.001$) compared to the initial values. 1 month after surgery, NT had a tendency to increase, but its values were lower than the initial level by 19.28% ($p < 0.05$). After 3 months, NT amounted to 4.71 ± 0.21 perf. units. and exceeded baseline by 31.88% ($p < 0.05$) (Fig. 6) (Table II). Myogenic tone (MT), which is also involved in the active regulation of microcirculation before dental implant surgery, was equal to 2.22 ± 0.10 perf. units. After dental implantation with immediate loading, after 3 days, the values tended to decrease, and after 10 days after surgery, they increased by 99.06% ($p < 0.001$).

One month after dental implantation with immediate loading, the values dropped and differed from the preoperative level by 10.97%, and by 24.24% - after 3 months ($p < 0.05$) (Fig. 7) (Table II).

The shunting index (SHI), which characterizes the ratio of shunting and nutritional blood flow in the microvascular network, was initially 0.62 ± 0.03 u.o. 3 days after dental implantation, his digital values tended to decrease (Fig. 8). After 10 days, its values increased by 2.85 times, which may indicate the reaction of the microcirculation in the mucous membrane to the traumatic injury of the jawbone during dental implantation and immediate intraoperative prosthetics ($p < 0.001$). One month after the operation, the SHI decreased and was higher from the initial level by 37.48% ($p < 0.05$), and after 3 months its values slightly differed from the preoperative level - 0.59 ± 0.03 u.o. This may indicate the restoration of blood circulation in the microcirculation system of the jawbones after dental implantation.

DISCUSSION

According to research János Jarabin, Zsófia Bere: the non-implanted yet previously operated contralateral sides of the patients demonstrated marginally lower ($p = 0.09$) blood flux index. The STR sides however, showed significantly lower (average 217 %) provoked blood flux compared to cotrols ($p < 0.001$). At the STP sides a maladaptation could be observed (average 316 %) compared to the contralateral sides ($p = 0.53$). STP sides demonstrated a significantly better blood flow improvement compared to the STR sides ($p = 0.02$). These results suggest a favorable postoperative condition of vascular microcirculation after STP, than after STR surgery [18].

According to research Vladimir Kokovic, Elena Krsljak: the measured PU values ranged from 17.5 to 130.7 with a mean of 53.05. LDF>60 PU was measured at 8 implants sites, LDF<60 and ≥ 40 PU at 18sites, and LDF<40 PU at 10 sites. There were no statistically significant differences between mean values of the left and right sides of the mandibles as well as between different implants positions. There was no statistically significant difference in vascularity between men (53.3) and women (51.5) [19].

According to research F F Losev , E K Krechina , M M Kayugin LDF data, it was established that the level of microcirculation in the mucous membrane of the alveolar ridge in response to the functional load after fixation of the removable prosthesis with fixation on locators (group 1) and on telescopic crowns (group 2), it is accompanied by an increase in the level of M and σ by 37% and 66%, and by 2.2-2.4, respectively, which indicated the development of hyperemia in the microcirculatory bed, which is associated with functional load and persists up to 3 and 6 months, respectively. Restoration of microcirculation occurs after 6 and 12 months, respectively, after prosthetics, depending on the supporting elements. After fixation of the removable prosthesis, SpO2 and U decreased, which characterized the development of hypoxia in the tissues due to functional load, which stopped after 3 and 6 months, respectively [20].

CONCLUSIONS

The use of laser Doppler flowmetry made it possible to monitor the microcirculation system during dental implantation and immediate intraoperative prosthetics. The obtained results indicate a pronounced reaction of microcirculation up to the 3rd day after surgery, an increase in blood perfusion of the mucous membrane by 2.7 times while maintaining vasomotor activity, which indicates adaptation to the injury and immediate loading of the denture in the postoperative period.

3 months after dental surgery and immediate intraoperative prosthetics, all indicators of microcirculation approach the initial values before surgery. This fact confirms the positive dynamics of recovery of microhemodynamics after dental implantation and immediate intraoperative prosthetics, thus, the dynamics of osseointegration is accelerated, which is confirmed by high indicators of the implant stability coefficient.

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Conflict of interest
The Authors declare no conflicts of interest.

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ORIGINAL ARTICLE

ADDITIONAL RISKS ARISING IN THE PROCESS OF PROVIDING MEDICAL AID TO PATIENTS WITH COVID-19

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ABSTRACT

The aim: In order to assess the degree of transforming danger, for face masks, used in the providing respiratory support process to specialized department patients with varying degrees of the COVID-19 course severity, we conducted a series of bacteriological studies into an additional opportunistic bacteria reservoir. With the purpose of assessment of the face respiratory masks inner surface bacterial contamination intensity during their use to provide respiratory support to patients with COVID-19.

Materials and methods: A bacteriological study of the inner surface of 60 disposable individual face respiratory masks was carried out at different times of providing respiratory support to patients with COVID-19.

Results: It is shown that during use, the inner surface of the respiratory mask is colonized by staphylococci and gram-negative opportunistic bacteria. With increasing time of the mask using, the density of colonization of its inner surface increases.

Conclusions: In the process of long-term non-invasive lung ventilation and oxygen therapy for patients with COVID-19, the inner surface of face respiratory masks is colonized with opportunistic bacteria, which creates the risk of contamination by the latter of the pathologically changed lung parenchyma and the addition of secondary bacterial infection.

KEY WORDS: non-invasive lung ventilation, opportunistic bacteria, sensitivity to antibiotics

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INTRODUCTION

Infectious complications of bacterial etiology associated with the medical care provision remain an unsolved problem of modern medicine, which negatively affects the indicators of treatment efficiency. Among them, nosocomial pneumonias (NP) of bacterial etiology occupy the second most frequent place after urinary tract infections. The specific weight of nosocomial pneumonias in the overall structure of infectious complications in intensive care unit patients reaches 25%, and the mortality rate ranges from 30% to 70%. The development of NP prolongs the patient's stay in the hospital by an average of 7-9 days and leads to a significant increase in the cost of treatment [1, 2].

The risks of NP are especially high in patients requiring respiratory support using artificial ventilation. The frequency of NP in patients undergoing mechanical ventilation is 9-21% [3-5]. At the same time, elements of the respiratory circuit most often become the reservoir of pathogens of NP. It has been proven that a few hours after the start of ventilation, the surface of the inhalation tube begins to be covered with a bacterial biofilm [6, 7].

The risks of the complications related to the provision of medical care increasing frequency and their negative consequences have increased many times since the beginning of the COVID-19 pandemic. After all, according to WHO estimates, the epidemic has covered all continents, including Antarctica, and the number of people infected with this infection has already crossed the mark of 0.5 billion people [8, 9]. About 15% of those infected with COVID-19 require inpatient treatment and, given the high risk of severe respiratory distress, almost all of those hospitalized need non-invasive lung ventilation and oxygen therapy using face masks [10-12].

In economically developed countries with a high level of medical care, the rules of hospital hygiene are strictly regulated by special regulations, the observance of which in the process of providing non-invasive respiratory support minimizes the risks of bacterial infections in patients with viral pneumonia. However, in the conditions of a fast and large flow of serious patients entering the department, significant overloading of medical personnel, these standards may be violated. Then there is a danger of the opportunistic bacterial microflora additional reservoirs formation and the

emergence of new risks of complicating the course of the main disease [13, 14].

In order to assess the degree of transforming danger, for face masks, used in the providing respiratory support process to specialized department patients with varying degrees of the COVID-19 course severity, we conducted a series of bacteriological studies into an additional opportunistic bacteria reservoir.

THE AIM

The aim of the work was to assessment of the face respiratory masks inner surface bacterial contamination intensity during their use to provide respiratory support to patients with COVID-19.

MATERIALS AND METHODS

A bacteriological study of the 60 disposable individual face respiratory masks inner surface was carried out at different times of providing respiratory support to patients with COVID-19. Among them, 19 patients used a mask for 1-4 days (group I), 21 patients for 5-9 days (group II), 12 patients for 10-19 days (group III), and 8 patients for 20 or more days of hospital stay and use of respiratory support (group IV).

The material for bacteriological culture was collected by wiping the inner surface of the mask in the locus of the oral cavity projection with a disposable cotton applicator pre-moistened with a sterile isotonic sodium chloride solution. The applicator was placed in a test tube with a transport medium and delivered to the bacteriological laboratory within 2 hours.

The studied material was sown using the semi-quantitative cup method [15] on Columbia agar with 5% lamb blood and mannitol-salt agar (GRASO biotech, Poland). After incubation of the seeds in thermostatic conditions for a day, the number of colony-forming units of bacteria per cm^2 of the respiratory mask examined surface (CFU/cm2) was calculated. Species identification of microorganisms was carried out taking into account the morphological, tinctorial and cultural properties of microorganisms, the results of biochemical typing. Biochemical typing was performed on diagnostic panels STAfitest-24 and ENTEROtest-24 of PLIVA – Lachema a. s. Brno, Czech Republic.

The sensitivity of selected microorganisms' strains to antibiotics was studied using the standard disk diffusion method (DDM) in accordance with the requirements of EUCAST (European Committee on Antimicrobial Susceptibility Testing).

Methods of statistical analysis used in the study: In the statistical processing of the obtained results, we used generally accepted formulas: the arithmetic mean and the standard error of the arithmetic mean. The calculation of the statistical probability of the difference between two samples of the arithmetic mean and its standard error was performed according to the Student's t-test using special tables for absolute and relative values [16].

In addition, using the X2 method with the determination of Fisher's exact criterion for small samples, we studied the possibility of the duration of oxygen therapy influence in patients with COVID-19 on the appearance of microflora in oxygen masks, and also investigated the possibility of the duration of oxygen therapy influence on the appearance of resistant strains of microflora to antibiotics.

The correlation between the indicators of the number of CFU of microflora isolated from oxygen masks in patients with covid-19 and the duration of oxygen therapy was studied using the non-parametric Spearman method with the determination of the Spearman rank correlation coefficient (ρ).

To select the optimal threshold value for the duration of oxygen therapy associated with the occurrence of the risk of the microflora appearance in the oxygen mask in patients with COVID-19, an analysis of ROC (Receiver Operator Characteristic) curves was performed. With the help of ROC-analysis, a study of the sensitivity and specificity of determining the oxygen therapy duration was carried out, and the quality of the model was established in relation to the possibility of the determined duration of oxygen therapy prognostic use.

Statistical calculations were performed using the MedCalc®Software bvba computer program and the SPSS 12 software package (license number 9593869, owned by the Department of Infectious Diseases of the M.I. Pirogov Vinnytsia National Medical University) and "STATISTICA 5.5" using parametric and non-parametric methods of evaluating the obtained results. The latter belongs to the CMIT of National Pirogov Memorial Medical University, Vinnytsya, license number AXXR910A374605FA.

RESULTS

Analysis of the bacteriological studies results showed that in 45% of cases bacterial microflora is absent on the inner surfaces of respiratory masks. In the remaining cases, representatives of the *Staphylococcus* family were found in varying numbers. A total of 33 strains of microorganisms of this genus were isolated. Among them, 3 strains (9.1%) split mannitol on mannitol-salt agar, coagulated plasma and were assigned to the species *S. aureus* based on the set of biological charac-

teristics. Species identification of the remaining strains of staphylococci that do not coagulate plasma was not performed.

In three cases, gram-negative bacteria were isolated in association with non-coagulable plasma staphylococci. In one case, a culture of *E. cloacae* was isolated from the inner surface of the mask after 9 days of using it. *K. pneumoniae* bacteria were isolated from the surface of the mask used by the patient on the 18th day of hospital stay. In another case, after 20 days of use, the inner surface of the mask was found to be contaminated with *A. baumannii*. The density of bacterial colonization of the investigated surfaces by gram-negative microorganisms in all three cases was low (1×102 CFU/cm^2).

Analysis of the face masks inner surface contamination degree with staphylococci during different periods of their use is summarized in Table I. Listed in Table I, the results of bacteriological studies reflect a completely expected pattern of growth in the intensity of the medical product surface bacterial contamination with an increase in the period of the latter use.

According to the study results, we found that in the first group of patients with COVID-19 who received oxygen therapy, microflora was sown from breathing masks in 8 (42.1%) patients, in the second group - 11 (52.3%), in the third group - 7 (58.3%), IV – 7 (87.5%).

The comparative analysis revealed a significant increase in the number of patients in the IV group compared to the I ($p<0.01$) and II ($p<0.05$) groups in which the microflora was cultured from respiratory masks. However, we did not establish significant differences ($p>0.05$) between III and IV groups, as well as between I, II and III groups (Fig. 1).

Having conducted an analysis by the X2 method with the determination of Fisher's exact test for small samples, we established a significant influence ($p<0.01$) of the oxygen therapy duration in patients with COVID-19 with the appearance of microflora in oxygen masks only for the IV group when compared with the I group (X2 - 6.72). At the same time, for groups I, II and III, the duration of oxygen therapy in patients with COVID-19 did not affect ($p>0.05$) the detection of microflora in oxygen masks. When comparing groups I and II, X2 was 0.93; for groups I and III - 1.56; for II and III groups - 0.48; for II and IV groups - 4.7; for groups III and IV – 3.58, respectively.

In the group of patients who received respiratory support for no more than 4 days, no staphylococci were detected on the inner surface of the masks in more than half of the cases (57.8%). Cases of high degree surface contamination (1×106 and > CFU/cm2) were not observed in patients of this group. As the period of use increased, the specific weight of absence microflora cases on the mask surface decreased. Thus, when examined after 10 days of mask use, microflora was not cultured in only 41.6% of cases, and after 20 days - in 12.5% of examinations. Further detailed analysis of the indicators listed in the table.1 illustrates a pronounced trend of increasing the contamination density of the face masks inner surface with staphylococci as the term of their use increases.

It should be noted that among all cases of microflora detection in oxygen masks of patients with COVID-19 who received oxygen therapy, in 30 (90.9%) cases, *Staphylococcus* was cultured as a monoculture, and only in 3 (9.1%) cases two microorganisms were isolated at the same time: 1 *Staphylococcus* + *E. Cloacae* (II group), 1 *Staphylococcus* + *K. Pneumoniae* (III group) and 1 *Staphylococcus* + *A. baumannii* (IV group). The difference between groups regarding the detection of several bacterial strains in oxygen masks was not significant ($p>0.05$).

We analyzed the differences between the examined groups regarding the number of CFU of the microflora, which was seeded from oxygen masks in patients with COVID-19 receiving oxygen therapy. It was established that the number of CFU in patients of the 1st group was 2.17lg4±9.5lg3; II groups - 5.31lg6±4.8lg6 CFU; III – 2.52lg6±1.2lg6 CFU and IV group – 5.03lg6±1.8lg6 CFU.

When comparing the number of CFUs in groups I and III (2.17lg4±9.5lg3 vs. 2.52lg6±1.2lg6) and groups I and IV (2.17lg4±9.5lg3 vs. 5.03g6±1.8lg6) a significant increase in the number of CFU was observed in III ($p<0.05$) and IV ($p<0.01$), respectively. At the same time, there were no differences ($p>0.05$) in the number of CFU of microorganisms between I and II groups (2.17lg4±9.5lg3 vs. 5.31lg6±4.8lg6), II and III groups (5.31lg6±4, 8lg6 against 2.52lg6±1.2lg6), II and IV groups (5.31lg6±4.8lg6 against 5.03g6±1.8lg6), and between III and IV groups (2.52lg6±1.2lg6 against 5, 03g6±1.8lg6) respectively.

At the same time, we established a close correlation between the number of CFU of microflora cultured from oxygen masks in patients with COVID-19 and the oxygen therapy duration. Spearman's rank coefficient was 0.427 ($p = 0.0007$).

In order to evaluate the effectiveness of detecting microflora in oxygen masks in patients with COVID-19 who received oxygen therapy, as well as to determine the oxygen therapy term after which there is a possible risk of contamination with the oxygen mask microflora, we performed a ROC analysis of the obtained results. It was established that the best cut-off point for the duration of oxygen therapy associated with the occurrence of the risk of microflora in the oxygen mask > 6: sensitivity 63.6 (95% CI: 45.1 – 79.6), specificity 70.4 (95 % SI: 49.8-86.2). AUC 0.72±0.07; $p<0.004$. Thus, according to

Table I. Characteristics of the inner surface of face masks contamination intensity with staphylococci during different periods of use

Group of examined	Microbial contamination density (CFU/cm²) of the mask inner surface			
	0	1×10²-10³	1×10⁴-10⁵	1×10⁶ і >
	Number of cases in the group in %			
I	57,8	21,1	21,1	0
II	47,6	19,1	23,8	9,5
III	41,6	16,7	16,7	25,0
IV	12,5	12,5	25,0	50,0

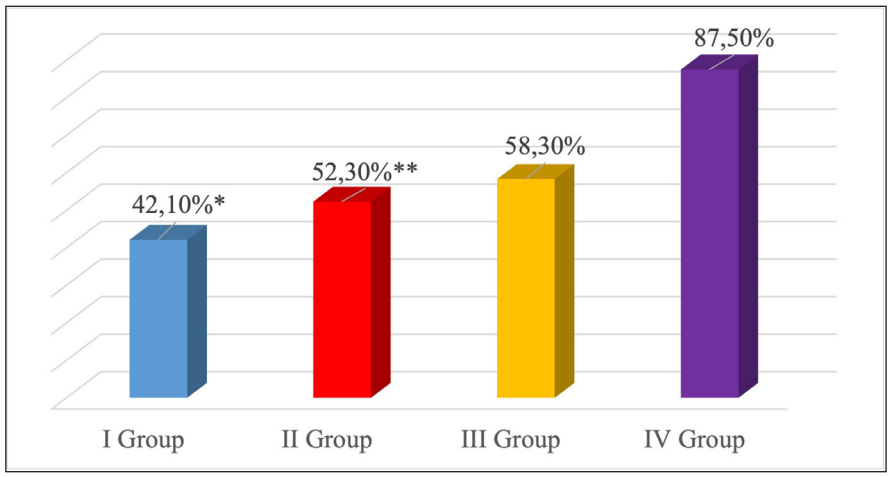

Fig. 1. Dynamics of microflora detection in breathing masks of a patients with COVID -19 who received oxygen therapy.
* - <0.01 compared to IV group;
** - <0.05 compared to IV group;

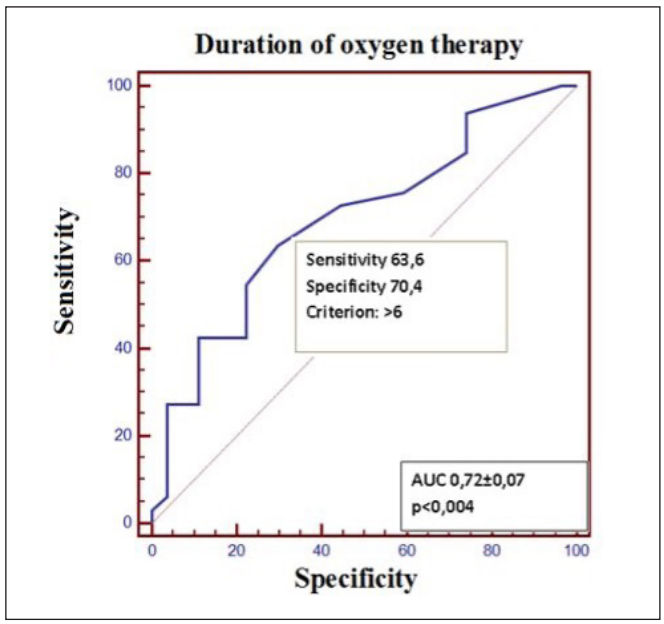

Fig. 2. ROC curve of the oxygen therapy duration assessment in patients with COVID-19, as a prognostic marker of the microflora appearance in masks for oxygen therapy.

the classification of ROC curve models, the proposed model for determining the infectivity of oxygen masks during oxygen therapy of patients with COVID-19 has good quality (Fig. 2).

The study of the sensitivity of the selected strains of staphylococci to antibiotics showed that most of them (22 out of 33) did not show polyresistance. They were sensitive to unprotected β-lactam structure antibiotics, aminoglycosides, macrolides, lincosamides, fluoroquinolones. The remaining strains (11) showed resistance to several different chemical groups antibiotics and belonged to multidrug-resistant (MDR) according to EUCAST standards.

The isolated strain of *E. cloacae* shows sensitivity to cephalosporins, aminoglycosides, fluoroquinolones, carbapenems and does not belong to polyresistant ones. *K. pneumonsae* and *A. baumannii* strains showed resistance to protected aminopenicillins, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, while maintaining sensitivity to polymyxin. According to the EUCAST classification, they are considered strains with extended resistance (HDR, extensively drug-resistant).

During the analysis of the sensitivity of cultured microorganisms to antibiotics, it was established that in group I, among all detected cases, resistance to antibiotics was observed in 1 (12.5%) case, in group II - 3 (27.3%) cases, in group III - 4 (57.1%) cases and in the IV group – 4 (57.1%) cases. We established a significant ($p<0.05$) increase in the bacteria antibiotic-resistant strains detection frequency in groups III and IV, respectively, compared to group I (Fig. 3). At the same time, there were no differences between I and II, II and III, II and IV groups, as well as between III and IV groups ($p>0.05$) (Fig. 3).

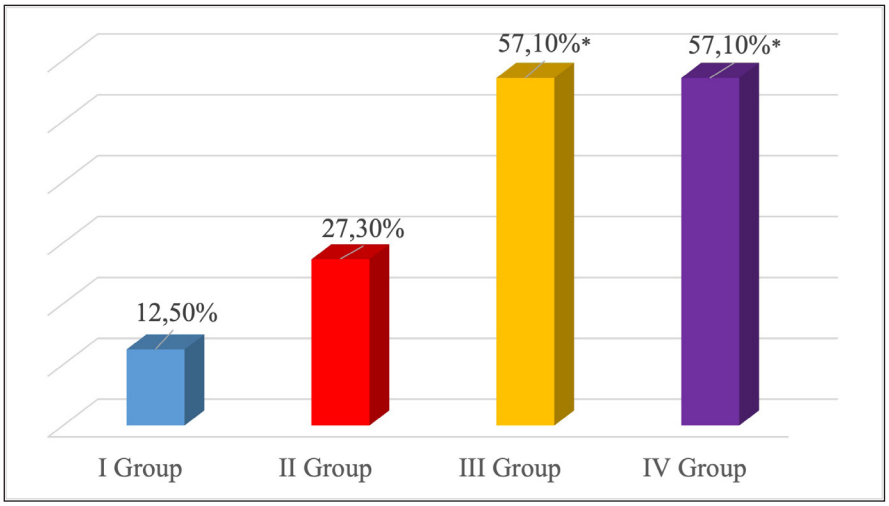

Fig. 3. Dynamics of detection of resistant microflora in respiratory masks of patients with Covid-19 who received oxygen therapy
* - <0.05 compared to the I group

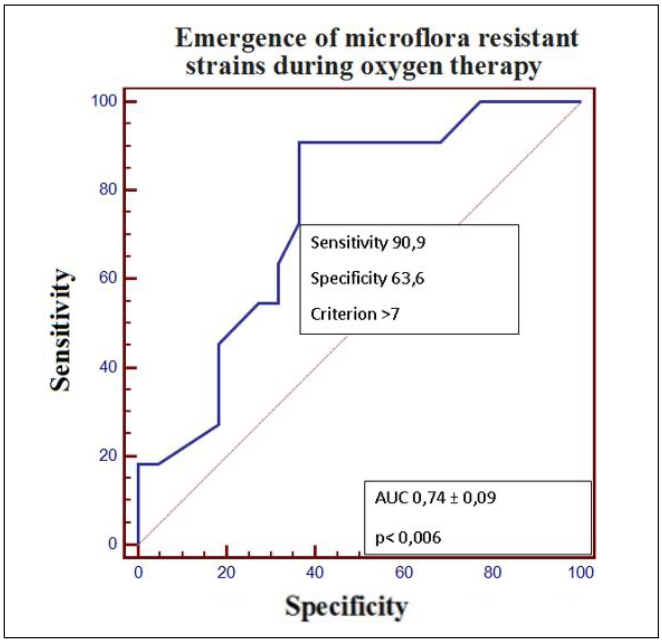

Fig. 4. ROC curve of the oxygen therapy duration assessment in patients with COVID-19 as a prognostic marker of the resistant microflora appearance in masks for oxygen therapy

In order to evaluate the effectiveness of detecting microflora resistant to antibiotics in oxygen masks in patients with Covid-19 who received oxygen therapy, as well as to determine the term of oxygen therapy after which the risk of the resistant microflora appearance in oxygen masks is possible, we performed a ROC analysis of the obtained results. It was found that the best cut-off point for the duration of oxygen therapy, when there is a risk of the resistant microflora appearance in the oxygen mask > 7: sensitivity 90.9 (95% CI: 58.7 – 99.8), specificity 63.6 (95 % SI: 40.7-82.8). AUC 0.74±0.09; p<0.006. According to the classification of ROC-curve models, the proposed model for determining the infection of oxygen masks with resistant microflora. during patients with COVID-19 oxygen therapy, has good quality (Fig. 4).

DISCUSSION

The use of face breathing masks in the process of providing respiratory support to patients with COVID-19 is a leading technological technique, since the use of nasal cannulae does not always ensure the target saturation indicators achievement. Masks are single-use polymer products for medical purposes. Given the individual use of these products, proper attention is not always paid to hygienic care of their condition, often even with their long-term use [17, 18].

The above results of the respiratory masks inner surfaces bacteriological studies during more or less long-term use in the process of non-invasive ventilation and oxygen therapy of patients with COVID-19 indicate that their polymer surface is a favorable substrate for the opportunistic bacteria adhesion. After all, in 42.2% of the examinations cases, conducted by us, the inner surface of the masks turned out to be colonized with staphylococci already during the first 4 days of using the product. With the increase in the period of the mask use, the density of staphylococci population increases. The presence of a sufficient amount of moisture, a stable temperature close to the optimum for bacterial growth creates favorable conditions for adhesion, reproduction and biofilm formation on the polymer surface of staphylococci, which belong to the normal flora of the human body and easily enter the respiratory circuit. Further maturation of biofilms, their dispersion and entry into air streams creates a real threat of dissemination of pathologically changed lung parenchyma by potentially dangerous bacteria [19-21].

It is indicative that as the period of the mask use increases, the frequency of its surface colonization with antibiotic-resistant variants of staphylococci increases, as well as the appearance of gram-negative bacteria among selected strains, which often take part in the development of hospital-acquired pneumonia. The belonging of these strains to MDR and even XDR variants suggests their hospital origin [22-25].

The established regularities indicate the need to pay more attention to the hygiene of respiratory face masks. Despite the individual nature of their use, in the process of long-term respiratory support for patients with COVID-19, a frequent change of the device is necessary, or, at the very least, regular disinfection of the inner surface with the help of antiseptic agents [26, 27].

CONCLUSIONS

In the process of long-term non-invasive lung ventilation and oxygen therapy for patients with COVID-19, the inner surface of face respiratory masks is colonized with opportunistic bacteria, which creates the risk of contamination by the latter of the pathologically changed lung parenchyma and the addition of secondary bacterial infection.

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ORIGINAL ARTICLE

THE EFFECT OF COGNITIVE-BEHAVIORAL THERAPY ON INDICATORS OF LIPID AND CARBOHYDRATE METABOLISM IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE ON THE BACKGROUND OF ABDOMINAL OBESITY

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ABSTRACT

The aim: Analyse the impact and effectiveness of cognitive-behavioural therapy in patients with NAFLD on the background of AO on indicators of lipid and carbohydrate metabolism.

Materials and methods: 85 patients with NAFLD and obesity of the 1st degree (42 women, 43 men) were examined. All patients were divided into two clinical groups: 1st group (research) - 48 people, who underwent a course of CBT for 6 months; the control group - 37 people, who followed only diet therapy, recommended physical activity, without involvement in CBT. The general clinical examination of patients consisted of the measurement of anthropometric and physiological indicators, the study of lipid and carbohydrate profile indicators.

Results: After a 6-month course of CBT, a decrease in the atherogenic coefficient (AC) was recorded in the patients of the 1st group in 2.29 times ($p=0.037<0.05$), the number of patients with hypertension of the 1st degree decreased in 1.8 times ($p=0.0047<0.05$) in comparison with the initial indicators (on the 1st day) and amount of patients with normal blood pressure of the 1st group increased by 2.4 times ($p=0.0115<0.05$) compared to the control group.

Conclusions: The course of CBT should be an integral component in the program for the management of obese patients to optimize not only the psycho-emotional condition, but also to correct lipid and carbohydrate metabolism indicators, reduce the risk of developing cardiovascular and neurological complications.

KEY WORDS: obesity, body mass index, non-alcoholic fatty liver disease, cognitive-behavioural therapy

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INTRODUCTION

Obesity for the last 30 years became important medical and social problem almost in all countries of the world. WHO is considering obesity as an epidemic in the world in 2012, 260 million patients are registered on obesity, and in less than 10 years constitutes about 500 million people, and is depicted the trend regarding the increase in the disease among young men and personal young age [1-3]. At present most of the time countries Western Europe and the USA on obesity 20-25 % of the population suffer ((BMI)>30 kg/m^2). Surplus mass bodies (BMI>25 kg/m^2) in industrial developed countries, except of Japan and China, has about half population. In many morbidities in the countries of the world on obesity in the middle increased by 2 times [4-7]. In Ukraine too noted growth numbers of persons with surplus mass bodies and obesity.

Excess weight and NAFLD always go hand in hand. Obesity leads to violation work of the liver, what appears hepatomegaly, an increase in biochemical liver indicators and histological ones changes (large-pored steatosis, fat hepatitis, fibrosis and cirrhosis), as well as non- alcoholic fatty liver dystrophy. The most characteristic the sign is an increase in liver enzymes (ALT and AST). From 40% to 100% of patients with non- alcoholic steatohepatitis, according to aggregate data several research, they have obesity [8-10]. In patients who are suffering on obesity, in ~ 75% of cases meets steatosis, steatohepatitis in~20% and liver cirrhosis in~2%. Although clinical, laboratory and histological signs non-alcoholic fat lesions of the liver have already been determined, the nature and pathogenesis this illness not at all until now understandable. In many illnesses flowing asymptomatic, or sick to complain for increased

fatigue, malaise or a feeling of discomfort in the area stomach. Hepatomegaly it is noted in 75% of patients [11-13]. AST /ALT ratio in patients is usually less than one, in contrast to the value this index in patients on alcoholic steatohepatitis. During observation patients for 1-7 years was noted progress liver disease in 40% of patients, and in 10% it developed cirrhosis. In countries with a wide distribution obesity is a pathology becomes one of the main causes of liver cirrhosis. To the same obesity increases the risk of development fibrosis and cirrhosis in alcoholic patients' liver damage and hepatitis C. The development of this pathology often associated with abdominal obesity, insulin resistance (increased glucose level on an empty stomach and insulin in the blood), diabetes, hypertriglyceridemia, low serum concentration of HDL and AH. Gradual weight loss maybe by 10% or more adjust the level of liver enzymes and help reduce the size of the liver, the content of fat in the liver fabric and weakening manifested steatohepatitis. But rapid weight loss after surgery treatment obesity, for with the help of a very low-calorie diet or fasting maybe to provoke inflammation or aggravation steatohepatitis [14].

Considering the large number of non-psychotic mental disorders, disorders of carbohydrate and lipid metabolism, which are often a component of this comorbid pathology, the problem of non-drug treatment in the form of cognitive-behavioural therapy is quite relevant.

THE AIM

Analyse the impact and effectiveness of cognitive-behavioural therapy in patients with NAFLD on the background of AO on indicators of lipid and carbohydrate metabolism.

MATERIALS AND METHODS

85 patients (42 women, 43 men) were examined on the basis of the University Clinic of the Black Sea National University named after Peter Mohyla and the polyclinic of the State University "Territorial Medical Association of the Ministry of Internal Affairs of Ukraine in Mykolaiv region". The age of women varied from 19 to 60 years (average age 41.5±11.2 years), the age of men - from 22 to 60 years (average age - 42.3±12.3 years). All patients were divided into two clinical groups: 1st group (research group) - 48 people (24 women and 24 men), patients with NAFLD and obesity of the 1st degree with BMI from 31.0 kg/m^2 to 34.1 kg/m^2. average BMI=32.55±1.07 kg/m^2, who underwent a course of CBT, diet therapy, and physical activity for 6 months; the control group - 37 people of NAFLD (19 women and 18 men) with abdominal obesity of the 1st degree (BMI from 30.0 kg/m 2 to 33.9 kg/m^2; average BMI=31.95±1.03 kg/m^2), patients who followed only diet therapy, recommended physical activity, without involvement in cognitive-behavioural training (information about obesity was obtained from brochures and mass media).

The criteria for including patients in the study were: patients with NAFLD on the background of overweight (BMI=25-29.9 kg/m^2) and alimentary obesity (exogenously-constitutional) obesity) of various degrees of severity (BMI>25 kg/m^2, OT>88 cm in women and > 102 cm in men, accumulation of adipose tissue in the waist area, around the navel, in the shape of an "apple"), lack of data on taking hormonal or medicinal drugs that could affect BMI.

Exclusion criteria from the study were: patients with genetically determined obesity against the background of multiple organ failure; patients with diseases of the hypothalamic-pituitary system, adrenal glands, hypothyroidism, and hypo ovarian obesity; cerebral obesity; dissemination of systemic lesions and infectious diseases; iatrogenic obesity against the background of taking drugs that contribute to an increase in body weight; the study also excluded patients with viral hepatitis B and C, patients who consumed alcohol in a dose of more than 30 g per day for men and 20 g per day for women), patients with congenital liver diseases and who used steatogenic drugs.

The general clinical examination of the patients consisted of the measurement of anthropometric and physiological parameters, the study of the lipid and carbohydrate profile.

The anthropometric study included: determination of body height and weight, calculation of the body mass index (kg/m^2), measurement of the circumference of the waist and hips, the index "waist circumference/hip circumference" (OT/OS); physiological: measurement of blood pressure (presence of arterial hypertension), heart rate.

Assessment of carbohydrate metabolism consisted of: determination of fasting blood glucose with a DIACONT glucometer followed by an oral glucose tolerance test (OGTT) (glucose – 1.75 g/kg of body weight, but not more than 75 g), measurement of glycosylated haemoglobin with a DCA Vantage TM analyser; the level of insulin in the blood with the Eleksys-2010 analyser, the calculation of the HOMA-IR index was carried out according to the formula: HOMA-IR=fasting insulin *fasting glucose/22.5.

For the analysis of lipid profile indicators (cholesterol, triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (LDL), high-density lipoprotein

(HDL), the LabAnalyt-PFS-30 lipid spectrum analyser was used; the atherogenic index (IA) was calculated according to the formula: IA=(CX-HDL)/HDL.

Patients who met the following criteria were included in the experiment: exclusion of alcohol consumption ≥30 g/day in the case of a man and ≥20 g/day in the case of a woman; individual and family anamnesis of diabetes, hypertension and cardiovascular diseases were analysed in detail, BMI, waist circumference, changes in body weight were calculated; laboratory-confirmed negative results for markers of HBV and HCV infection; excluding the use of steatogenic drugs; slight or moderate increase in the activity of ALT and AST (AST/ ALT <1) and GGT (≈50% of cases, according to ultrasound (increased echogenicity steatosis) of the liver, rarely hepatomegaly; in cirrhosis, symptoms of portal hypertension).

Patients of all clinical groups followed the recommendations for a healthy diet and physical activity. Balanced diet included: reducing the daily calorie content of food, in which the daily calorie content of food for women is 1200-1400 kcal, and for men - 1400-1600 kcal; the amount of fats used with food did not exceed 29% of the daily caloric intake of food, 30-50% of consumed fats consisted of polyunsaturated fatty acids, the source of animal fats was lean fish, poultry (without skin), lean beef tenderloin was occasionally allowed; the share of carbohydrates accounted for 50-60% of the number of kilocalories consumed daily, to enrich food with calcium in the diet it was recommended to introduce milk or kefir 0.5%, table salt was limited to 4.5 g per day. In the presence of violations of carbohydrate metabolism, it was also recommended to count bread unit, with obesity of the 1st degree (BMI=35-40 kg/m^2), the daily norm is -10 bread unit, obesity of the 2nd degree (BMI above 40 kg/m^2) - 6-8 bread unit).

Physical activity was recommended mainly due to aerobic exercise: patients with a BMI of up to 40 kg/m^2 - it was recommended to start with walking at an average pace - 100 steps per minute, for 30 minutes, 3-4 times a week, gradually increasing the pace of walking to a high (160 steps per minute), duration - up to 45-60 minutes, frequency - up to 1 time per day. 1st group (research) - 48 people (24 women and 24 men), patients with NAFLD and obesity of the 1st degree with an average BMI=32.55±1.07 kg/m^2, took an active part in CBT. The total number of meetings with patients was 2 times a week, the duration of one coaching session was 30 minutes, a total of 48 sessions. The components of CBT were: motivational interview; the stage of actual cognitive-behavioural training (psychoeducation); the final stage is consolidation of the acquired attitudes and prevention of withdrawal relapses. The main methods of CBT used during coaching trainings: cognitive psychotherapy, reciprocal inhibition, rational-emotional psychotherapy, self-control, "Stop-tap" method and anxiety control, relaxation, self-instruction, self-observation, research and analysis of threatening consequences, methods of finding advantages and disadvantages, paradoxical intention.

The effectiveness of CBT was carried out by comparing between group and intra-group indicators of lipid and carbohydrate metabolism on the 1st day, 180th day.

Statistical for Windows version 8.0 (License Number: 139-845-755) and computer program "Excel 2010" (Microsoft) were used for statistical data processing. At the first stage of the calculation, descriptive statistics were obtained for indicators measured on a quantitative scale. Such characteristics are median and average value as measures of position; standard deviation and quartiles as measures of dispersion; minimum and maximum value as indicators of sample size. The Kruskel-Wallis test was used to determine differences between independent groups using nonparametric statistical methods. The obtained results were considered statistically significant at $p<0.05$.

The study was conducted in accordance with the basic bioethical norms of the Helsinki Declaration of the World Medical Association "Ethical Principles of Medical Research Involving Humans as Research Subjects" (1964), with changes and additions to the General Declaration on Bioethics and Human Rights. United Nations (2005), Council of Europe Convention on Human Rights and Biomedicine (1997). All participants were informed about the goals, organization, methods of the study and signed an informed consent to participate in it. All measures are also taken to ensure patient anonymity.

RESULTS

The results of the study established a positive effect of CBT on anthropometric, physiological indicators, lipid and carbohydrate metabolism. Men and women responded equally to therapy.

In the patients of the 1st group, after a 6-month course of CBT, a decrease was recorded: total cholesterol (TC) by an average of 1.4 mmol/l ($p=0.045< 0.05$), triglycerides (TG) by 1.92 mmol/l ($p=0.039<0.05$), low-density lipoproteins (LDL-C) by 1.86 mmol/l ($p=0.048<0.05$), very low density lipoproteins (VLDL-C) by 2.73 mmol/l ($p=0.049<0.05$); an increase in high-density lipoproteins (HDL-C) by 1.92 mmol/l ($p=0.019<0.05$), compared to the final indicators of the control group. The most positive effect of CBT was recorded on the atherogenicity coefficient (AC), which was reduced by 2.29 times

Table I. Average lipidogram indicators in clinical groups, depending on the type of corrective therapy

Lipidogram indicators	1st group (research), BMI average = 32.55 ±1.07 kg/m2 (n =48)			Control group, BMI average =31.95±1.03 kg/m2 (n =37)			Difference between 1st group and control group	
	Therapy: CBT + diet + exercise			Therapy: Diet + exercise				
	Indicators before CBT (1st day)	Indicators after 6 months of CBT (180th day)	The difference in lipid metabolism indicators before and after CBT in the 1st group	Indicators before CBT (1st day)	Indicators after 6 months (180th day)	The difference between initial and final indicators of lipid metabolism in the control group	Distinction between initial indicators lipid exchange in 1st and control the group	Distinction between final indicators lipid exchange in 1st and control the group
	M±SEM	M±SEM	P	M±SEM	M±SEM	P	P	P
TC, mmol/l	7.77 ± 0.24	5.55 ± 0.70	0.046*	7.69 ± 0.3	6.91 ± 0.69	0.020**	0.075	0.045****
TG, mmol/l	3.86 ± 0.40	2.01 ± 0.39	0.003*	3.87 ± 0.4	3.71 ± 0.44	0.017**	0.914	0.039****
HDL-C, mmol/l	0.79 ± 0.09	1.52 ± 0.40	0.041*	0.91 ± 0.12	0.91 ± 0.06	0.718	0.021***	0.048****
LDL-C, mmol/l	5.99 ± 0.43	3.23 ± 0.49	0.035*	5.97 ± 0.44	5.76 ± 0.34	0.0179**	0.668	0.049****
VLDL-C, mmol/l	2.02 ± 0.33	0.75 ± 0.11	0.031*	1.73 ± 0.49	1.51 ± 0.28	0.021**	0.006	0.019****
AC, mmol/l	8.92 ± 1.20	2.91 ± 1.28	0.047*	7.58 ± 1.36	6.65 ± 0.98	0.031**	0.043***	0.037****

1.*- changes are probable with indicators before treatment in patients of the 1st group (p<0.05); 2.** - changes are probable with indicators before treatment in patients of the control group (p<0.05); 3. *** - presence of intergroup difference in lipidogram indicators on the 1st day of therapy (p<0.05); 4**** - presence of intergroup difference in lipidogram indicators on the 180th day of therapy (p<0.05).

(p=0.047<0.05) among patients of the 1st group, unlike patients of the control group, which indicates a significant reduction in the risk of developing cardiovascular and neurological complications in obese patients, who underwent a course of CBT, unlike patients of the control group (table I).

After a course of CBT, the patients also tended to decrease in carbohydrate metabolism. In patients of the 1st group, a decrease in fasting blood glucose by 0.5±0.72 mmol/l (p=0.0006<0.05)) was reliably recorded after the 6-month course of therapy, compared to the initial values; data of postprandial glucose also decreased by 0.85±0.84 mmol/l (p=0.032<0.05), when comparing initial and final indicators; HbA1c decreased by 0.99±0.57 mmol/l (p=0.041<0.05), in people of the 1st group after a course of CBT. The difference between the initial and final indicators of carbohydrate metabolism among 1st group was recorded in all indicators of carbohydrate metabolism, which indicates a significant decrease in glycaemia indicators in patients after the course of CBT, in comparison with the control group (table II).

In patients of the 1st group after CBT (on the 180th day of observation), the number of patients with hypertension of the 1st degree decreased by 1.8 times (p=0.0047<0.05) in comparison with the initial indicators (on the 1st day). The number of patients with normal blood pressure on the 180th day of observation in the subjects of the 1st group increased by 2.4 times (p=0.0115<0.05) compared to the control group. In the control group, no statistically significant differences could be established during the entire observation period (all p >0.05) (table III).

Patients who received complex therapy managed to improve anthropometric indicators. A decrease in body weight from 6 to 11 kg in patients of the 1st group was registered 2.2 times more often (p-value=0.0286<0.05) than among the control group; and body weight loss in the range from 18 to 23 kg was recorded 3.62 times more often (p-value=0.0004<0.05) than in patients of the control group without CBT (table IV).

Decrease in waist circumference within 8-9 cm on the 180th day of observation in the 1st group was registered 1.5 times more often (p-value=0.0045<0.05) than in the control group (table V).

Decrease in hip circumference within 6-7 cm within 6 months. observation was registered 2.36 times more often (p-value=0.0038<0.05) among patients of the 1st group than among the control group; a decrease in thigh volume indicators of more than 10 cm in patients of the 1st group was also recorded 2.36 times more often (p-value=0.0371<0.05) than in the control group (table VI).

Table II. Indicators of carbohydrate metabolism in clinical groups, depending on the type of corrective therapy

State indicators of carbohydrate metabolism	1st group (research), BMI = 32.55 ±1.07 kg/m², (n=48)			Control group, BMI = 31.95±1.03 kg/m², (n=37)			Difference between 1st and Control groups	
	Therapy: CBT + diet + exercise			Therapy: Diet + exercise				
	Indicators before CBT (1st day)	Indicators after 6 months of CBT (180th day)	The difference in carbohydrate metabolism indicators before and after CBT in the 1st group	Indicators before CBT (1st day)	Indicators after 6 months (180th day)	Differences in carbohydrate metabolism indicators in the control group	The difference between the initial indicators of carbohydrate metabolism in the 1st and the control group	The difference between the final indicators of carbohydrate metabolism in 1st and the control group
	M±SEM	M±SEM	P	M±SEM	M±SEM	P	P	P
Fasting glycemia, mmol /l	6.13 ± 0.72	5.63 ± 0.57	0.0006	6.28 ± 0.71	6.67 ± 0.45	0.0189**	0.4041	0.045***
Glucose level after 2 hours, after consuming 75 g of sugar -containing solution, mmol/l	8.09 ± 1.31	7.24 ± 0.37	0.032*	9.03 ± 1.38	9.08 ± 0.66	0.8965	0.0001	0.039 ***
HbA1c	6.50 ± 0.73	5.51 ± 0.4	0.041 *	6.53 ± 0.57	6.23 ± 0.37	0.0143**	0.8108	0.038 ***
Insulin , U/l	15.46 ± 4.74	14.36 ± 2.09	0.1275	12.85 ± 4.68	11.92 ± 1.79	0.4841	0.0086	0.040***
NOMA-IR	4.00 ± 1.56	3 . 57 ± 0.69	0.1471	3.63 ± 1.27	3.54 ± 0.62	0.7457	0.3059	0.762

1.*- changes are probable with indicators before treatment in patients of the 1st group ($p<0.05$); 2.** - changes are probable with indicators before treatment in patients of the control group ($p<0.05$); 3. *** - presence of intergroup difference in carbohydrates indicators on the 180th day of therapy ($p<0.05$).

Table III. Indicators of blood pressure (BP) in clinical groups, depending on the type of corrective therapy

Indicators of blood pressure (mm Hg) (M±SEM)	1st group (research), $BMI_{avg.}$= 32.55 ±1.07 kg/m², (n=48)			Control group, $BMI_{average}$=31.95±1.03 kg/m², (n=37)			Difference between 1st and Control groups	
	Therapy: CBT + diet + exercise			Therapy: Diet + exercise				
	Indicators before CBT (1st day)	Indicators after 6 months of CBT (180th day)	The difference in blood pressure indicators before and after CPT in the 1st group	Indicators before CBT (1st day)	Indicators after 6 months (180th day)	The difference between initial and final BP values in the control group	The difference between the initial blood pressure indicators in the 1st and the control group	The difference between the final blood pressure indicators in 1st and the control group
	n (%)	n (%)	P	n (%)	n (%)	P	P	P
<120/80	2 (4.2%)	8 (16.6%)	0.0909	3 (8.1%)	7(18.9%)	0.3081	0.6488	0.7832
121-139/81-89	6 (12.5%)	19 (39.6%)	0.0047 *	5(13.5%)	8(21.6%)	0.5426	1.0000	0.0115**
140-159/90-99	20 (41.7%)	11 (22.9%)	0.0499*	13(35.1%)	10(27%)	0.6160	0.6546	0.8006
160-179/100-109	12 (25%)	6 (12.5%)	0.1902	10(27%)	8(21.6%)	0.7870	1.0000	0.3772
>180/110	8 (16.6%)	4 (8.4%)	0.3553	6(16.3%)	4(10.9%)	0.7355	1.0000	0.7235

1.*- changes are probable with indicators before treatment in patients of the 1st group ($p<0.05$); 2.** - presence of intergroup difference in BP indicators on the 180th day of therapy ($p<0.05$).

Table IV. The difference in the intergroup indicators of the patient's weight (kg), depending on the type of corrective therapy

The amount of lost weight, on the 180th day of therapy, (kg)	1st group (research), $BMI_{avg.}$= 32.55 ±1.07 kg/m², (n=48)			Control group	Difference between groups $BMI_{average}$=31.95±1.03 kg/m², (n=37)		
	Therapy: CBT + diet + exercises			Therapy: Diet + exercises			
	M±SEM	n	%	M±SEM	n	%	p-value
0-5	4.0±1.0	3	6.7	3.67± 0.82	6	16.7	0.1687
6-11	7.2 ± 1.64	5	11.1	9.00±1.48	11	28.9	0.0286*
12-17	14.2 ± 2.28	5	11.1	15.22±1.72	9	26.7	0.1388
18-23	19.41 ± 1.24	29	57.8	20.88±2.17	8	23.3	0.0004*
24-29	26.75 ± 1.89	4	8.9	24.00±0.02	2	3.3	0.6927
>30	30.0 ± 0.01	2	4.4	30.00±0.03	1	1.1	1.0000

* The presence of an intergroup difference in anthropometric indicators on the 180th day of treatment ($p < 0.05$).

Table V. The difference in the intergroup indicators of the waist circumference (cm) of the patients depending on the type of therapy (on 180th day)

Waist circumference (cm), on the 180th day of observation	1st group (research), BMI_{avg}.= 32.55 ±1.07 kg/m², (n=48)			Control group, $BMI_{average}$ =31.95±1.03 kg/m², (n=37)			Difference between 1st and Control groups
	Therapy: CBT + diet + exercises			Therapy: Diet + exercises			
	M±SEM	n	%	M±SEM	n	%	p-value
0-1	0.55 ± 0.07	2	5.6	0.7 ± 0.21	5	16.7	0.2312
2-3	2.53 ± 0.21	3	8.9	2.4 ± 0.42	8	21.1	0.0511
4-5	4.62 ± 0.38	5	10.0	4.47 ± 0.29	9	22.2	0.1388
6-7	6.50 ± 0.31	6	11.1	6.27 ± 0.25	3	8.9	0.7253
8-9	8.43 ± 0.35	26	53.3	8.31 ± 0.29	9	22.2	0.0045 *
>10	10.22 ± 0.15	6	11.1	10.13 ± 0.15	3	8.9	0.7253

* The presence of an intergroup difference in anthropometric indicators on the 180th day of treatment ($p < 0.05$).

Table VI. Intergroup difference in hip circumference (cm) depending on type of therapy

Hip circumference (cm), on 180th day therapy	1st group (research), BMI_{avg}.= 32.55 ±1.07 kg/m², (n=48)			Control group, $BMI_{average}$ =31.95±1.03 kg/m², (n=37)			Difference between 1st and control groups
	Therapy: CBT + diet + exercises			Therapy: Diet + exercises			
	M±SEM	n	%	M±SEM	n	%	p-value
0-1	0.55 ± 0.07	2	4.5	0.75 ± 0.23	6	20.0	0.0734
2-3	2.53 ± 0.21	3	5.5	2.42 ± 0.40	5	18.9	0.2867
4-5	4.45 ± 0.64	2	4.4	4.00 ± 0.01	2	7.8	1.0000
6-7	6.68 ± 0.30	33	67.8	6.49 ± 0.19	14	41.1	0.0038*
8-9	8.47 ± 0.48	6	13.3	8.63 ± 0.15	3	10.0	0.7253
>10	10.35 ± 0.07	2	4.5	10.31 ± 0.34	7	2.2	0.0371*

* The presence of an intergroup difference in anthropometric indicators on the 180th day of treatment ($p < 0.05$).

DISCUSSION

Despite the fact that the pathogenesis of mechanisms of the development and treatment of obesity are well known, clinical observations indicate the problem of recurrent weight gain by patients after the end of the treatment course [11-13]. An alternative for preventing the recurrence of obesity and long-term weight maintenance is the implementation of cognitive-behavioural therapy. The results of our study demonstrated improvements in indicators of fat and lipid metabolism. Moreover, CBT had a more pronounced effect on fat metabolism than on carbohydrate metabolism. In our opinion, this is due to the fact that thanks to the course of CBT under the supervision of a coaching doctor, it was possible to change the stereotypes of eating behaviour, develop motivation and increase the patient's physical activity. Many factors (stress, watching television, emotional exhaustion, or, conversely, a state of joy, etc.) can be associated with overeating, which demonstrates the so-called behavioural chain. Breaking the links within the chain (for example, recommending that patients eat only in the kitchen or dining room, thereby breaking the link between eating and watching TV) will reduce the power of each trigger. The absence of side effects, unlike the use of drug treatment, a stable result, the possibility of lifestyle changes and modification of eating behaviour, which can be achieved by this method, make CBT the most rationally justified and alternative method in the treatment of obesity, especially at the early stage of treatment.

CONCLUSIONS

1. After a 6-month course of CBT, KA decreased in 2.29 times (p=0.037<0.05) among patients of the 1st group, unlike patients of the control group, which indicates a significant reduction in the risk of developing cardiovascular and neurological complications in obese patients, who underwent a course of CBT, unlike patients in the control group.
2. In patients of the 1st group after CBT (on the 180th day of observation), the number of patients with hypertension of the 1st degree decreased by 1.8

times ($p=0.0047<0.05$) in comparison with the initial indicators (on the 1st day) and amount of patients with normal blood pressure increased in 2.4 times ($p=0.0115<0.05$) compared to the control group.

3. The difference between the final indicators of carbohydrate metabolism among 1st and the control group was recorded in all indicators of carbohydrate metabolism, which indicates a significant decrease in glycaemia indicators in patients after the course of CBT, in comparison with the control group.
4. The CBT course helps to change the stereotypes of eating behaviour, develop motivation and increase the physical activity of the patient; therefore it is an alternative method not only for preventing the recurrence of obesity and long-term weight maintenance, but also for correcting lipid and carbohydrate indicators and reducing the risk of developing cardiovascular complications.

PRACTICAL SIGNIFICANCE AND PROSPECTS FOR FURTHER RESEARCH

The main problem of the modern health care system is a rapid increase in the number of cases of obesity, relapse and ineffectiveness of long-term maintenance of the desired weight, the development of non-psychotic mental disorders and metabolic disorders, as the main component of obese patients. By the fact that most leading health care specialists continue to consider and correct obesity only from the perspective of somatic pathology, forgetting the role of psychological factors and the need for primary impact on the patient's psycho-emotional sphere. The results of our research have demonstrated the possibility of correcting cognitive-behavioural therapy, not only for physiological, neuropsychiatric disorders, but also to influence the patient's carbohydrate and lipid metabolism. Therefore, the use of CBT is an alternative treatment for this comorbid pathology and an alternative to drug therapy at an early stage of treatment.

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ORIGINAL ARTICLE

MULTIFACTORIAL REGRESSION MODEL FOR PREDICTING THE LEVEL OF HEAT SENSITIVITY IN HEALTHY YOUNG PEOPLE IN THE CONTEXT OF GLOBAL WARMING

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ABSTRACT

The aim: To create a mathematical model for predicting the level of heat sensitivity in healthy young people based on multivariate regression analysis.

Materials and methods: 150 healthy young people aged 17-20 years answered the questionnaire "Levels of heat sensitivity", underwent a heat test and mathematical analysis of the heart rate, after which the results were used to build a regression model of heat sensitivity.

Results: The model of mathematical prediction of heat sensitivity (CHSL1/CHSL2), which we proposed for the first time, takes into account the most significant factors that influence the determination of higher and lower sensitivity to heat (Q1-Q6, %LF2, %HF1, %HF2, HR1, HR2), so its use will allow timely identification of individuals who are particularly susceptible to the effects of elevated ambient temperature and prevent the development of potential negative consequences of this exposure.

Conclusions: Based on the results obtained, it is possible to use this prognostic model in the future to develop a diagnostic system for determining the level of heat sensitivity.

KEY WORDS: global warming, heat sensitivity, cardiovascular system, multivariate regression analysis, individual sensitivity, climate change

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INTRODUCTION

The World Health Organisation (WHO) has identified modern climate change as one of the greatest threats to human health in the 21st century [1]. The average annual temperature in Ukraine and Europe has increased by almost 1.5 °C over the past 30 years [2]. Global warming poses a great danger to life on planet Earth, and if this problem is neglected and the necessary measures are not taken, society will face catastrophic consequences [3, 4].

It is known that the negative impact on human life and health is caused not by the increase in air temperature, but by the resulting increase in the load on the cardiovascular system, which causes disorders of its activity and functioning of the body as a whole, and limits the adaptive potential of the human body and thermoregulatory mechanisms [5 - 7].

Scientific studies have shown that people have individual sensitivity to various environmental factors [8]. The degree of vulnerability to climate change is modulated by the age of the individual, the environmental conditions in which he or she lives, the activity of the cardiovascular system, the presence or absence of chronic diseases, mental disorders, and access to healthcare [9, 10].

Given the above, it is important to predict heat sensitivity to identify people with a higher sensitivity to elevated ambient temperatures, i.e. those most vulnerable to climate change, and to actively monitor their health status, increase their adaptive potential and stress resistance [11 - 14].

Today, multivariate regression analysis is one of the most common and accurate methods for predicting the occurrence of any disease or pathological condition, which can be used as secondary prevention and facilitate the work of practitioners [15]. In our work, we propose to use a similar approach to determine the individual response of the human body to thermal changes in the environment, as well as to build an appropriate mathematical model for prediction. This will allow timely identification of individuals with

higher heat sensitivity in order to prevent or minimise the negative impact of global warming on their bodies.

THE AIM

To create a mathematical model for predicting the level of heat sensitivity in healthy young people based on multivariate regression analysis.

MATERIALS AND METHODS

To develop a mathematical model for predicting the level of heat sensitivity, 150 healthy young people aged 17-20 years were examined. The study was conducted on the basis of the laboratory of psychophysiological research certified by the Ministry of Health of Ukraine at the Department of Physiology with the basics of bioethics and biosafety of the I.Y. Gorbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (Certificate No. 003/18). The study was conducted in compliance with bioethical standards (Minutes of the meeting of the Bioethics Commission of I. Gorbachevsky Ternopil National Medical University No. 73 of 03 April 2023). All subjects signed an informed consent to the study.

The level of heat sensitivity was determined by the following algorithm [16]:

1. At the first stage, the subjects were asked to answer the questions of the questionnaire «Levels of heat sensitivity» (copyright certificate No. 115529 of 01.11.2022) issued by State Enterprise "Ukrainian Intellectual Property Institute" (UIPI) [17]. The number of points obtained was used to evaluate the result: 0-6 - lower heat sensitivity, 7-16 - higher.
2. The next step in the study was a heat test. Before it began, the temperature in the room was measured using an electronic thermometer «Omron Gentle Temp 720 (MS-720-E)». The subjects' pulse rate and blood pressure were palpated using the auscultatory method using the MMP-60 device, after which the hands of both hands

Table I. Potential factors that may influence the level of heat sensitivity (CHSL1/CHSL2) and their indexation in young healthy people.

№	Names of the CHSL1/CHSL2 heat sensitivity level predictors	Symbols in the model
1.	Question in questionnaire №1.	Q1
2.	Question in questionnaire №2.	Q2
3.	Question in questionnaire №3.	Q3
4.	Question in questionnaire №4.	Q4
5.	Question in questionnaire №5.	Q5
6.	Question in questionnaire №6.	Q6
7.	Question in questionnaire №7.	Q7
8.	Question in questionnaire №8.	Q8
9.	The autonomous balance coefficient is the ratio of the power of low-frequency and high-frequency waves in the heart rhythm to the heat test.	LF\HF1
10.	Autonomous balance coefficient - the ratio of low-frequency to high-frequency wave power in the heart rhythm after a heat test.	LF\HF2
11.	The relative value of low-frequency wave power in % in the heart rhythm before the heat test.	%LF1
12.	The relative value of low-frequency wave power in % in the heart rate after the heat test.	%LF2
13.	Power of the spectrum of the high-frequency component of variability in % of the total power of oscillations before the heat test.	%HF1
14.	Power of the spectrum of the high-frequency component of variability in % of the total power of oscillations after the heat test.	%HF2
15.	Stress index (mode amplitude/(2 × mode × variation range)) to the heat test, units.	SI1
16.	Stress index after heat test, unit.	SI2
17.	Heart rate, beats per minute, before the heat test.	HR1
18.	Heart rate, beats per minute, after the heat test.	HR2
19.	Systolic blood pressure, mm Hg, before the heat test.	SBP1
20.	Systolic blood pressure, mm Hg, after the heat test.	SBP2
21.	Diastolic blood pressure, mm Hg, before heat test	DBP1
22.	Diastolic blood pressure, mm Hg, after heat test	DBP2

were immersed in a container of warm water (45 °C) for 3 minutes. During this, at the 2nd minute, the pulse rate and blood pressure were determined. The same procedure was repeated after the hands were removed from the water and every 2 minutes until the pulse rate and blood pressure were restored and the time was recorded. According to the results of the heat test, it was found that in people with higher heat sensitivity, after the test, the pulse rate and blood pressure increased, while in people with lower heat sensitivity, the pulse rate decreased or did not change.

3. All subjects underwent mathematical analysis of the heart rhythm using the computer software complex «Spectrum+» (Kharkiv, Ukraine). 5-minute segments of rhythmograms were recorded. Statistical and spectral methods, as well as cardiac intervalography according to R.M. Baevsky were used. The results of heart rate variability showed that parasympathetic influences prevail in individuals with lower heat sensitivity, and in subjects with higher heat sensitivity, the activity of the sympathetic division of the autonomic nervous system prevails.

To build a mathematical model for predicting the level of heat sensitivity, 150 subjects were included in the study with different levels of sensitivity to the thermal factor: lower (n=94) and higher (n=56). After that, we analysed 22 possible probable factors (Table I) that may affect the level of heat sensitivity (CHSL). In the prognostic model, the higher heat sensitivity is designated as CHSL1, and the lower - CHSL2, and, accordingly, the dependence of the heat sensitivity coefficient (CHSL1/ CHSL2) on the studied factors was established.

The statistical processing of the data and the construction of a regression model of the level of heat sensitivity were carried out using the programs «Microsoft Excel 2016» and «Statistica 10.0» (StatSoft. Inc.). The model for predicting the value of the heat sensitivity coefficient included 11 factors with correlation coefficients ranging from 0.3 to 0.7. The total initial number of predictors was 22. The factors of CHSL1/CHSL2 occurrence with a significance level of $p>0.05$ were excluded from the regression analysis. To check the quality of the prognostic model, the Neigelkerk criterion (R2) was used, and to assess the model's acceptability, ANOVA was performed.

RESULTS AND DISCUSSION

To assess the significance of the influence of the above factors (Table I) on the occurrence of heat sensitivity (CHSL1/ CHSL2), a stepwise multivariate regression analysis was performed.

At the initial stage, a correlation matrix was constructed, which confirms the absence of pairwise correlation coefficients greater than 0.7, i.e. multicollinear factors.

Then, to build a regression model, we included 22 potential triggers of CHSL1/CHSL2. The regression coefficients «b» (Beta) were calculated, which show for each individual predictor the ratio of influence on the degree of CHSL1/ CHSL2 in the subjects (Fig. 1).

Factors Q7, Q8, LF/HF1, LF/HF2, %LF1, SI1, SI2, DBP1, SBP2, DBP2, with a significance level of $p>0.05$, were excluded from further analysis. Since the significance levels of the remaining 12 risk factors were $p<0.05$, they were included in the mathematical model for predicting the occurrence of CHSL1/CHSL2.

After processing the potential 22 factors using multivariate regression analysis, the following predictors with a p value of 0.05 were included in the mathematical prediction model (Q1-6, %LF2, %HF1, %HF2, HR1, HR2). It was found that the significance level of SBP1 was $p>0.05$, so this factor was also not included in the construction of the prognostic model of heat sensitivity (CHSL1/CHSL2) (Fig. 2).

Fig. 3 shows the result of obtaining the 11 studied factors, which were found to be the most significant for predicting the level of heat sensitivity (CHSL1/CHSL2) and were used to build a multivariate regression model: Q1-6: questions 1-6 of the questionnaire; %LF2 - relative value of low-frequency wave power in % in the heart rate after the heat test; %HF1 - power of the spectrum of the high-frequency component of variability in % of the total power of oscillations before the heat test; %HF2 - power of the spectrum of the high-frequency component of variability in % of the total power of oscillations after the heat test; HR1 - heart rate, bpm, before the heat test; HR2 - heart rate, bpm, after the heat test (Table I).

Based on the results shown in Fig. 3, we build a mathematical model to determine the coefficient of occurrence of the heat sensitivity level (CHSL1/CHSL2):

CHSL1/CHSL2=-0,259210*Q1-0,147279*Q2-0,229916*Q3-0,082847*Q4-0,071384*Q5-0,072407*Q6-0,002567*(%LF2)-0,008002*(%HF1)+0,008610*(%HF2)+0,004509*HR1-0,005476*HR2

To assess the quality of the regression model, it was necessary to analyse the residual deviations, in particular, to obtain their histogram (Fig. 4). As can be seen from the histogram, the residual deviations are distributed symmetrically, approaching the curve of the normal distribution of residuals, so the statistical hypothesis about their distribution in accordance with the normal distribution law is not rejected.

In order to further confirm the residual deviations from the normal distribution law, a normal-probability graph was constructed (Fig. 5). Analysing its data, we note the absence

N=150	Regression Summary for Dependent Variable: CHSL1/CHSL2 (1 in 11) R= ,98824191 R?= ,97662208 Adjusted R?= ,97257236 F(22,127)=241,16 p<0,0000 Std.Error of estimate: ,08037					
	b*	Std.Err. of b*	b	Std.Err. of b	t(127)	p-value
Intercept			2,270676	0,140634	16,14599	0,000000
Q1	-0,231246	0,028058	-0,231246	0,028058	-8,24171	0,000000
Q2	-0,148823	0,019697	-0,125317	0,016586	-7,55576	0,000000
Q3	-0,210996	0,022136	-0,204185	0,021421	-9,53182	0,000000
Q4	-0,119503	0,024364	-0,070803	0,014435	-4,90489	0,000003
Q5	-0,092464	0,022897	-0,059137	0,014644	-4,03818	0,000093
Q6	-0,053025	0,018572	-0,064945	0,022747	-2,85515	0,005026
Q7	0,001749	0,014939	0,003563	0,030427	0,11708	0,906978
Q8	-0,025346	0,020156	-0,018867	0,015004	-1,25748	0,210886
LF\HF1	-0,039649	0,042568	-0,008128	0,008726	-0,93142	0,353404
LF\HF2	0,021628	0,042878	0,006395	0,012677	0,50441	0,614845
%LF1	0,066043	0,036993	0,003639	0,002038	1,78532	0,076596
%LF2	-0,113734	0,039094	-0,005360	0,001842	-2,90922	0,004279
%HF1	-0,232299	0,065753	-0,007147	0,002023	-3,53292	0,000574
%HF2	0,262540	0,066915	0,007998	0,002039	3,92346	0,000142
SI1	0,032920	0,039988	0,000230	0,000279	0,82324	0,411915
SI2	-0,059743	0,042595	-0,000342	0,000244	-1,40258	0,163183
HR1	0,090679	0,023288	0,004907	0,001260	3,89375	0,000159
SBP1	0,133403	0,042190	0,005477	0,001732	3,16199	0,001960
DBP1	-0,064162	0,037164	-0,003730	0,002161	-1,72645	0,086699
HR2	-0,099331	0,024049	-0,005688	0,001377	-4,13041	0,000065
SBP2	-0,068058	0,034968	-0,003085	0,001585	-1,94629	0,053828
DBP2	-0,022418	0,033364	-0,001333	0,001984	-0,67194	0,502841

Fig. 1. The result of obtaining significant factors for predicting the occurrence of CHSL1/CHSL2 in multivariate regression analysis in Statistica 10.0.

N=150	Regression Summary for Dependent Variable: CHSL1/CHSL2 (1 in 11) R= ,98566232 R?= ,97153021 Adjusted R?= ,96903650 F(12,137)=389,59 p<0,0000 Std.Error of estimate: ,08540					
	b*	Std.Err. of b*	b	Std.Err. of b	t(137)	p-value
Intercept			2,282238	0,139345	16,3783	0,000000
Q1	-0,259337	0,028260	-0,259337	0,028260	-9,1768	0,000000
Q2	-0,175130	0,019105	-0,147468	0,016087	-9,1668	0,000000
Q3	-0,237588	0,021360	-0,229919	0,020671	-11,1228	0,000000
Q4	-0,139799	0,023810	-0,082827	0,014107	-5,8715	0,000000
Q5	-0,111302	0,023069	-0,071185	0,014754	-4,8247	0,000004
Q6	-0,059041	0,018370	-0,072313	0,022500	-3,2140	0,001633
%LF2	-0,054182	0,020241	-0,002553	0,000954	-2,6769	0,008339
%HF1	-0,259747	0,053471	-0,007992	0,001645	-4,8577	0,000003
%HF2	0,282164	0,059033	0,008596	0,001798	4,7798	0,000004
HR1	0,083751	0,022194	0,004532	0,001201	3,7736	0,000239
SBP1	-0,001395	0,016155	-0,000057	0,000663	-0,0863	0,931334
HR2	-0,096291	0,023245	-0,005514	0,001331	-4,1425	0,000060

Fig. 2. The result of obtaining significant factors for predicting the occurrence of CHSL1/ CHSL2 in multivariate regression analysis in Statistica 10.0 without factors Q7, Q8, LF/HF1, LF/HF2, %LF1, SI1, SI2, DBP1, SBP2, DBP2.

N=150	Regression Summary for Dependent Variable: CHSL1/CHSL2 (1 in 11) R= ,98566153 R?= ,97152866 Adjusted R?= ,96925920 F(11,138)=428,09 p<0,0000 Std.Error of estimate: ,08509					
	b*	Std.Err. of b*	b	Std.Err. of b	t(138)	p-value
Intercept			2,274752	0,108682	20,9304	0,000000
Q1	-0,259210	0,028120	-0,259210	0,028120	-9,2181	0,000000
Q2	-0,174905	0,018858	-0,147279	0,015880	-9,2748	0,000000
Q3	-0,237585	0,021283	-0,229916	0,020597	-11,1629	0,000000
Q4	-0,139832	0,023721	-0,082847	0,014054	-5,8949	0,000000
Q5	-0,111614	0,022703	-0,071384	0,014520	-4,9163	0,000002
Q6	-0,059117	0,018283	-0,072407	0,022393	-3,2335	0,001530
%LF2	-0,054466	0,019900	-0,002567	0,000938	-2,7370	0,007018
%HF1	-0,260067	0,053151	-0,008002	0,001635	-4,8930	0,000003
%HF2	0,282628	0,058577	0,008610	0,001785	4,8249	0,000004
HR1	0,083308	0,021515	0,004509	0,001164	3,8720	0,000166
HR2	-0,095633	0,021880	-0,005476	0,001253	-4,3707	0,000024

Fig. 3. The result of obtaining significant factors for predicting the occurrence of CHSL1/CHSL2 in multivariate regression analysis in Statistica 10.0 without the SBP1 factor.

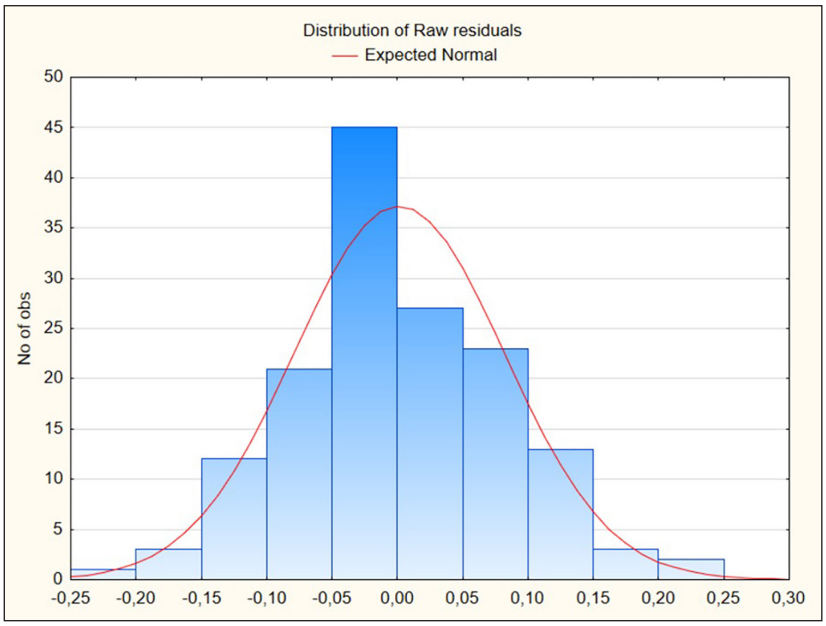

Fig. 4. Histogram of residual deviations of the multivariate regression model for predicting the occurrence of CHSL1/CHSL2.

of systematic deviations from the normal probability line. This allows us to conclude that the residual deviations are distributed according to the normal distribution law.

The next step was to assess the acceptability of the model as a whole, for which we performed an ANOVA analysis (Fig. 6). Analysing the data obtained, we can conclude that the model for predicting the level of heat sensitivity (CHSL1/CHSL2) is highly acceptable in general using ANOVA analysis, since the significance level is $p<0.001$, and the model itself will work better than a simple prediction using average values.

To further evaluate the quality of the mathematical model of heat sensitivity level (CHSL1/CHSL2), we analysed the coefficient of determination of the Neijelkerk (R2), which shows how much of the factors are taken into account in the prediction. It is considered a universal measure of the relationship between one random variable and others. The coefficient of determination varies from 0 to 1. The closer its value approaches «1», the better the multivariate regression model is. In the proposed CHSL1/CHSL2 mathematical model, the coefficient of determination is R2=0.9715 (in Statistica 10.0, R2= .97152866 (Fig. 4)). Thus, in our case, 97.15 % of the factors are taken into account in the model for predicting the level of heat sensitivity (CHSL1/CHSL2). The coefficient of determination indicates the

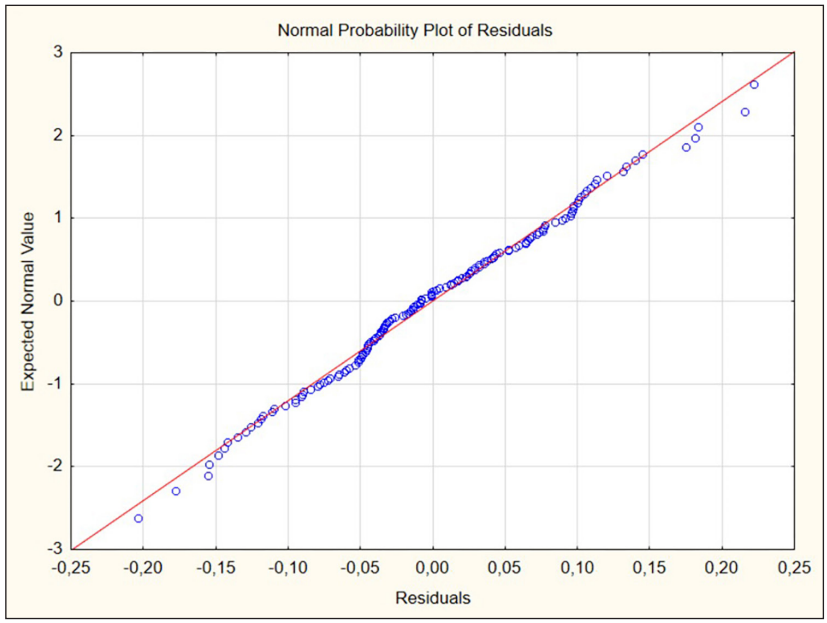

Fig. 5. Normal probability plot of residual deviations of the multivariate regression model for predicting the level of heat sensitivity (CHSL1/CHSL2).

Analysis of Variance; DV: CHSL1/CHSL2 (1 in 11)

Effect	Sums of Squares	df	Mean Squares	F	p-value
Regress.	34,09418	11	3,099471	428,0890	0,00
Residual	0,99915	138	0,007240		
Total	35,09333				

Fig. 6. Analysis of the coefficient of determination of the multivariate regression model for predicting CHSL1/CHSL2.

extent to which the experimental results confirm the mathematical model.

Thus, the prognostically significant factors affecting the level of heat sensitivity obtained by mathematical modelling (Q1-Q6, %LF2, %HF1, %HF2, HR1, HR2) confirmed that, first of all, to determine heat sensitivity, it is necessary to test according to the questionnaire «Levels of heat sensitivity», and then to conduct a heat test, In particular, a significant diagnostic criterion here is the heart rate before and after heat exposure, and the final stage, to confirm higher and lower heat sensitivity, is to assess the tone of the autonomic nervous system using mathematical analysis of the heart rate, where the power of the spectrum of high-frequency and low-frequency components of variability as a % of the total power of oscillations is important.

The model of mathematical prediction of heat sensitivity (CHSL1/CHSL2), which we have proposed for the first time, takes into account the most significant factors that influence the determination of higher and lower heat sensitivity, so its use will allow us to identify people who are particularly susceptible to high ambient temperatures and prevent the development of potential negative consequences of this exposure in time. This could become one of the stages of primary and secondary prevention of cardiovascular diseases in the future, reducing morbidity and mortality from circulatory system diseases, respectively.

Based on the results obtained, it is possible to use this prognostic model to develop a diagnostic system for determining the level of heat sensitivity in the future.

In future studies, it is also possible to conduct a ROC analysis to determine the sensitivity, specificity and accuracy of the proposed mathematical model for predicting the level of heat sensitivity (CHSL1/CHSL2).

CONCLUSIONS

1. The model of mathematical prediction of heat sensitivity (CHSL1/CHSL2), which we have proposed for the first time, takes into account the most significant factors that influence the occurrence of higher and lower heat sensitivity in healthy young people, so its use will allow timely identification of individuals susceptible to high ambient temperatures to prevent the development of potential negative effects of global warming.
2. Based on the results obtained, it is possible to use the mathematical prognostic model presented by us in the future to develop a diagnostic system for determining the level of heat sensitivity.

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ORIGINAL ARTICLE

ANTIOXIDANT-PROOXIDANT BALANCE OF THE KIDNEYS IN RATS OF DIFFERENT AGES UNDER CONDITIONS OF EXPERIMENTAL CRANIOSKELETAL TRAUMA

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ABSTRACT

The aim: To determine the peculiarities of the antioxidant-prooxidant balance in the kidney of rats of different ages under conditions of experimental cranioskeletal trauma (CST).

Materials and methods: The experiments involved 147 male white Wistar rats of different age groups. The first experimental group included immature animals aged 100-120 days. The second group included sexually mature animals aged 6-8 months. The third group included old animals aged 19-23 months. In all experimental groups, CST was modelled under thiopental-sodium anaesthesia. The control groups of rats was only injected with thiopental-sodium anaesthesia. The animals were withdrawn from the experiments under anaesthesia after 1, 3, 7, 14, 21 and 28 days by total bleeding from the heart. The content of reagents to thiobarbituric acid and catalase activity was determined in a 10 % kidney homogenate extract, and the antioxidant-prooxidant index (API) was calculated from the ratio of these two parameters.

Results: As a result of the application of CST in rats of different age groups, a decrease in the value of renal API was observed with a maximum in immature rats - after 7 days, in mature and old rats - after 14 days. By day 28, the index increased in all experimental groups, but did not reach the control level. The degree of decrease in renal API in old rats under the influence of CCT was significantly higher than in other experimental groups. In immature rats, the impairment of renal API after the application of CST was less, indicating higher reserve capacity of the renal antioxidant defence system in this age group of rats.

Conclusions: Simulation of CST in rats of different age groups is accompanied by a decrease in the value of API, which by day 28 does not reach the control level in any of the experimental groups. The degree of decrease in renal API value statistically significantly increases with increasing age of rats at all times of the post-traumatic period.

KEY WORDS: traumatic brain injury, skeletal injury, kidney, oxidative stress

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INTRODUCTION

One of the characteristic features of the third millennium is the epidemics of traumatism. As a result of the increase in the frequency of high-energy injuries, the proportion of multiple and combined injuries, in which several injuries occur simultaneously within the same or different anatomical areas, has significantly increased in the structure of modern trauma [1]. Today, such injuries are the main cause of mortality among people of working age, but according to forecasts, in the near future, mortality from trauma will take a dominant position among victims of all age groups [2].

Severe multiple and combined trauma is accompanied by circulatory shock with a significant deterioration in the blood supply to organs and tissues distant from the site of direct injury due to systemic haemodynamics disorders [3]. Under these conditions, the kidneys suffer the most, as they are physiologically characterised by a high level of blood supply and oxygen consumption [4].

Clinical observations have shown that successful recovery from shock prevents the victim from dying in a few days from various complications that are not directly related to the trauma [5]. It has become apparent that severe trauma causes secondary damage to internal organs, which is prolonged [6] and, if not treated adequately, leads to the development of multiple organ failure. The latter, in the case of a combined trauma, becomes the main cause of death in the post-shock period [7].

Among the combined trauma, cranioskeletal trauma (CST) stands out, characterised by a particularly severe course and high mortality [8]. The development of

traumatic shock along with impaired neuroendocrine regulation in the setting of CST has an adverse effect on the condition of tissues and organs with deepening hypoxia and increased generation of reactive oxygen species. At the same time, the antioxidant defence system is activated to compensate for antioxidant deficiency and maintain antioxidant-prooxidant homeostasis. Prolonged exposure to pathogenic factors of severe trauma leads to a disruption of the antioxidant-prooxidant balance in the internal organs towards increased free radical processes. Activation of free radical oxidation of lipids and cell membrane proteins causes an increase in their permeability and loss of membrane-dependent functions. It has been proven that the peak of free radical processes coincides with the maximum formation of proinflammatory mediators and is the trigger for the development of multiorgan dysfunction and failure [9].

However, the balance of lipid peroxidation and antioxidant defence in the kidney, depending on age, has not been studied, which formed the basis of this study.

THE AIM

To determine the peculiarities of antioxidant-prooxidant balance in the kidney of rats of different ages under conditions of experimental cranioskeletal trauma.

MATERIALS AND METHODS

The experiments involved 147 white male Wistar rats of different age groups selected by random method and kept on a standard vivarium diet. The first experimental group included immature animals aged 100-120 days and weighing 90-110 g. The second group included mature animals aged 6-8 months and weighing 180-200 g. The third group included old animals aged 19-23 months and weighing 300-320 g.

In all experimental groups (49 animals each), under thiopental-sodium anaesthesia (40 mg·kg^{-1}), CST was modelled according to the method described in [10]. In immature (young) animals, a dosed mechanical blow was applied sequentially to one of the thighs with a solid object with a wedge-shaped nozzle and an energy of 0.320 J, a closed fracture of one femur was achieved, and then a dosed blow to the skull with an energy of 0.226 J was applied with an object with a blunt end at a point 3 mm anterior to the interaural line. In sexually mature (adult) animals, hip fracture was modelled by applying a dosed mechanical damage to the hip with a wedge-shaped impactor with an energy of 0.637 J, which caused a closed fracture of the femur. Traumatic brain injury was caused by a dosed blow to the skull at a point 5 cm anterior to the interaural line with an energy of 0.375 J. In old rats, hip fracture was achieved by applying a dosed blow to the hip with an energy of 0.796 J, and traumatic brain injury was caused by a dosed blow to the skull with a blunt object with an energy of 0.549 J at a point 6 mm anterior to the interaural line.

The impact energy caused moderate traumatic brain injury in animals of different age groups. Animals with penetrating skull injuries or open hip fractures were not used in the experiments. In the control groups (7 rats each), animals were only put under thiopental-sodium anaesthesia.

The rats were withdrawn from the experiments under anaesthesia after 1, 3, 7, 14, 21 and 28 days by total bleeding from the heart. The cooled and blood-free liver was homogenised in a Silent Crasher 75000 homogeniser (Germany). The content of thiobarbituric acid reagents and catalase activity was determined in a 10 % kidney homogenate extract according to the methods described in [11] using a LabAnalyt SP-V1000 spectrophotometer (Granum, China). The ratio of the content of reagents to thiobarbituric acid to catalase activity was used to determine the antioxidant-prooxidant index (API), which indicates the anioxidant-prooxidant balance of the kidney [12].

The experiments were performed in accordance with the «General Ethical Principles for Experiments on Animals» adopted by the First National Congress on Bioethics (Kyiv, 2001) and agreed with the provisions of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986), as well as the conclusion of the Bioethics Commission of the Ternopil National Medical University named after I. Gorbachevsky of the Ministry of Health of Ukraine No. 72 of 06.01.2023.

The obtained digital material was processed in the STATISTICA software package (StatSoft Inc., USA). The median (Me), lower and upper quartiles (LQ, UQ) were determined. For an independent comparison of the degree of deviation of indicators in animals of different age groups, the average ratio of individual values of the studied indicators to the average value of the control group was calculated [13]. The significance of differences was assessed by the nonparametric Mann-Whitney test.

RESULTS

Studies have shown that in control groups of rats of different age groups, the value of renal API increased with age (Table 1), and in mature and old rats the index was statistically significantly higher than in immature rats

Table I. The value of API (units) under the influence of cranioskeletal trauma in rats of different ages, Me (LQ;UQ) - median (lower and upper quartiles)

Animal group	Counter-role	Period after injury					
		1 day	3 days	7 days	14 days	21 days	28 days
Group 1 Sexually immature	0,33 (0,31; 0,36)	0,34 (0,30; 0,35)	0,19^{*1} (0,17 0,21)	0,12^{*1,3} (0,12; 0,13)	0,10^{*1,3} (0,09 0,11)	0,16^{*1,7,14} (0,14 0,18	0,18^{*1,7,14} (0,17 0,22)
Group 2 Sexually Mature	0,52 (0,49; 0,54)	0,31* (0,27; 0,35)	0,16^{*1} (0,14; 0,17)	0,15^{*1} (0,14; 0,15)	0,091^{*1,3,7} (0,087; 0,093)	0,14^{*1,14} (0,13; 0,15)	0,20^{*1,7,14,21} (0,18; 0,21)
Group 3 Old	0,56 (0,50; 0,62)	0,17* (0,15; 0,19)	0,12^{*1} (0,11; 0,13)	0,092^{*1,3} (0,097; 0,101)	0,069^{*1,3,7} (0,062; 0,078)	0,11^{*1,14} (0,10; 0,11)	0,13^{*7,14,21} (0,13; 0,15)
p_{1-2}	<0,05	>0,05	>0,05	<0,05	>0,05	>0,05	>0,05
p_{1-3}	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
p_{2-3}	>0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05

Notes. Here and in other tables:
1.* - differences in relation to the control group are statistically significant (p<0.05);
2. p1-2 - significance of differences between study groups 1 and 2;
3. p1-3 - significance of differences between study groups 1 and 3;
4. p2-3 - significance of differences between study groups 2 and 3.

(by 57.6 and 68.4 %, respectively, $p_{1-2}< 0.05$, $p_{1-3}< 0.05$). The differences between the control groups of mature and old rats were not statistically significant ($p_{2-3}>0.05$).

As a result of simulated CST in the kidney of rats of all experimental groups, a decrease in the value of API was observed compared with the control (Table I). In immature rats, the result was statistically significant starting from day 3 of the experiment (by 42.4 % compared with the control, p< 0.05). The indicator reached a minimum after 7 days, became 36.8% lower than in the previous observation period (p< 0.05) and remained at the same level until day 14 (p> 0.05). At this time, the index was 69.7% lower than in the control group (p< 0.05). Subsequently, an increase in the value of renal API was noted. After 21 days, the index exceeded the result of 14 days by 60.0% (p< 0.05), after 28 days - by 80.0% (p< 0.05), but at this time it remained 45.4% lower than in the control group (p< 0.05).

In the group of sexually mature rats, the value of renal API became significantly lower than in the control group already 1 day after the application of CST (by 40.4 %, p< 0.05). Subsequently, the index decreased and reached a minimum value after 14 days of the experiment. At this time, the value of renal API was 82.5 % lower than in the control (p< 0.05) and compared to the results of 1, 3 and 7 days of the posttraumatic period (by 70.6, 43.1 and 39.3 %, respectively, p< 0.05). Subsequently, the index increased and after 21 days it was 53.8% higher than the result of 14 days (p< 0.05), and after 28 days - 2.20 times higher (p< 0.05). During this period, the value of renal API also became significantly higher compared to the results of the 3rd and 7th days of the experiment (by 25.0 and 33.3%, respectively, p< 0.05), but remained statistically significantly lower compared to the results of the 1st day of the experiment and control (by 35.4 and 61.5%, respectively, p< 0.05).

In the group of old rats, the value of kidney API was significantly lower than in the control group already 1 day after the application of CST (by 69.6 %, p< 0.05). Subsequently, the index decreased and reached its minimum value after 14 days of the experiment. At this time, the index was 87.7 % lower than in the control (p< 0.05) and compared to the results of the previous observation periods (by 59.4, 42.5 and 25.0 %, respectively, p< 0.05). After 21-28 days, an increase in the value of the studied indicator was noted compared to the result of the 14th day of the experiment - by 59.4 and 88.4 %, respectively (p< 0.05). After 28 days, the index also became significantly higher than after 7 days of the experiment (by 41.3 %, p< 0.05), but remained 76.8 % lower than in the control group (p< 0.05).

Comparison of the experimental groups showed that after 1, 3, 14, 321 and 28 days, the value of API in the experimental groups of immature and mature rats was significantly higher than in the group of old rats ($p_{1-3}< 0.05$, $p_{2-3}< 0.05$). However, after 14 days of the experiment, the value of the studied index was significantly higher in sexually mature rats - by 25.0% compared to immature rats ($p_{1-2}< 0.05$) and by 63.0% compared to old rats ($p_{2-3}< 0.05$). It should be noted that in old rats, the index was the lowest ($p_{1-3}< 0.05$).

Taking into account the different levels of API in control rats of different sexes, in order to independently compare the degree of deviation under the influence

Table II. Dynamics of the average ratio of individual values of kidney API to the average value of the control group under the influence of cranioskeletal trauma in rats of different ages, Me (LQ;UQ) - median (lower and upper quartiles)

Animal group	Period after injury					
	1 day	3 days	7 days	14 days	21 days	28 days
Group 1 Sexually immature	1,02 (0,91; 1,05)	0,57 (0,53; 0,62)	0,36 (0,35; 0,38)	0,32 (0,27; 0,32)	0,49 (0,41; 0,55)	0,55 (0,50; 0,66)
Group 2 Sexually mature	0,60 (0,51; 0,67)	0,31 (0,26; 0,32)	0,28 (0,26; 0,29)	0,17 (0,17; 0,18)	0,28 (0,25; 0,30)	0,39 (0,35; 0,40)
Group 3 Old	0,30 (0,27; 0,34)	0,21 (0,20; 0,23)	0,17 (0,16; 0,18)	0,12 (0,11; 0,14)	0,19 (0,18; 0,20)	0,23 (0,23; 0,26)
p_{1-2}	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
p_{1-3}	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
p_{2-3}	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05

of CCT, the average ratio of individual values of kidney API to the average value of the control group was additionally calculated (Table II). The table shows that the value of the studied index at all observation periods was statistically significantly lower in the group of old rats compared with immature and mature rats ($p_{1-3}< 0.05$, $p_{2-3}< 0.05$). Comparison of the groups of mature and immature rats showed that the index was statistically significantly lower in mature rats.

DISCUSSION

The results obtained indicate that the application of CST in rats of different age groups resulted in a decrease in renal API, which indicates the dominance of prooxidant mechanisms over antioxidant ones. On the one hand, this indicates the leading role of lipid peroxidation and depletion of the enzyme link of antioxidant defence, in particular catalase activity, in the pathogenesis of systemic disorders after application of CCP, which does not depend on age. On the other hand, the data obtained confirm the high sensitivity of the kidneys to hypoxia, which occurs as a result of the development of hemodynamic shock and the effects of free radicals that penetrate the systemic bloodstream from the area of direct damage to the brain and skeletal bones. As shown in [14], a decrease in API in the setting of CST prevails in the medulla of the kidney, which is particularly sensitive to hypoxia, leading to impaired renal function, in particular, renal sodium ion transport [15].

The maximum decrease in renal API under the influence of CST depends on age and occurs in immature rats after 7 days, in mature and old rats - after 14 days. By day 28, the index increases in all experimental groups, but does not reach the control level. Significant differences in the average ratio of individual values of kidney API to the average value of the control group are noteworthy, which depends on age and at all times of the posttraumatic period statistically significantly decreases from immature to old rats. The obtained results indicate that the degree of decrease in renal API values in old rats under the influence of CST is significantly greater than in other experimental groups. Thus, the depletion of the enzymatic link of antioxidant defence occurs more rapidly in old rats than in rats of other age groups. This may be a key pathogenetic factor in the development of acute kidney injury with a decrease in their functional capacity [14]. There is evidence that the presence of acute kidney injury is associated with a marked increase in mortality in patients with traumatic brain injury compared to patients without acute kidney injury [16]. In this regard, it can be assumed that a decrease in renal API may be one of the earliest indicators of oxidative stress in the kidneys and the worsening of their functional impairment.

At the same time, in immature rats, the value of renal API was lower after the application of CST, indicating higher reserve capacity of the renal antioxidant defence system in this age group of rats, which probably reduces the likelihood of developing secondary renal dysfunction.

The conducted studies prove the role of age in the intensity of renal antioxidant-prooxidant balance disorders as one of the systemic manifestations of CST. Different age-related resistance to prooxidant pathogenic factors of traumatic injury indicates the importance of an age-based approach to the choice of antioxidant therapy, which requires further in-depth preclinical study.

CONCLUSIONS

1. Modelling of CST in rats of different age groups is

accompanied by a decrease in renal API with a maximum of disorders after 7 days of post-traumatic period in the group of immature rats and after 14 days - in the group of mature and old rats with a subsequent increase, which by 28 days does not reach the control level in any of the experimental groups.

2. The amplitude of renal API disturbances under the influence of CST depends on age. In the dynamics of the post-traumatic period, the degree of decrease in renal API statistically significantly increases with increasing age of rats at all times of the post-traumatic period.

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ORIGINAL ARTICLE

FEATURES OF AFFECTIVE SYMPTOMS IN COMBATANTS WITH NON-PSYCHOTIC MENTAL DISORDERS THAT HAVE SUFFERED FROM COVID-19

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ABSTRACT

The aim: To establish the level of anxiety and depressive disorders and the impact of covid-19 on affective pathology in combatants with non-psychotic mental disorders who have suffered from COVID-19 in a comparative aspect.

Materials and methods: 252 male combatants with non-psychotic mental disorders who suffered from COVID-19 were clinically examined using HRDS and HARS scales.

Results: A lower prevalence of anxiety or fear was found in combatants who did not have COVID-19: 70.5% vs. 80.8%; low mood – 55.3% vs. 66.7%; emotional lability – 44.7% vs. 58.3%; irritability – 40.9% vs. 55.0%; emotional sensitivity – 53.0% vs. 71.7%; dulling of emotions – 6.8% vs. 6.7%; anhedonia – 77.3% vs. 83.3% of patients, rapid fatigue – 51.5% vs. 65.8%, feelings of guilt, futility, anxiety or fear – 78.0% vs. 87.5%, dissomnia – 47.0% vs. 61.7%; inability to concentrate and make decisions – 25.8% vs. 43.3%; thoughts of death or suicide – 25.0% vs. 35.8% pessimism – 21.2% vs. 31.7%, low self-esteem – 21.2% vs. 31.7%, unstable appetite and weight loss-17.4% vs. 24.2%. The overall HDRS depression score in combatants with non-psychotic mental disorders who did not have COVID-19 was also significantly lower: 15.29±4.16 points vs. 18.05±4.29 points. Similar patterns were found for indicators of anxiety on the HARS scale: 20.52±7.14 points vs. 24.53±6.69 points.

Conclusions: Combatants with non-psychotic mental disorders are characterized by high levels of depressive and anxiety disorders. COVID-19 disease aggravates the course of affective pathology in combatants with non-psychotic mental disorders and increases the incidence of their depressive and anxiety symptoms

KEY WORDS: combatants, COVID-19, non-psychotic mental disorders

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INTRODUCTION

Military conflicts have large-scale negative consequences for the mental health of their participants, whose spectrum includes reactive states, post-traumatic disorders, anxiety and depressive manifestations, the consequences of traumatic brain and somatic injuries, as well as the activation of suicidal and addictive behavior [1 – 4]. Changes in the psyche of combatants are complex in nature and are accompanied by manifestations of pronounced socio-psychological maladaptation, which determines the need for simultaneous therapeutic influence on psychopathological symptoms, the restoration of normal social functioning and the reduction of socio-psychological maladaptation [5 – 9]. Pathological changes in the psyche of combatants include not only reactive states, but also persistent patho-characterological transformations, as well as impaired cognitive functioning supported by psychological mechanisms of displacement, projection, regression, denial, dissociation, and compensation [10 – 12]. Combatants returning to peaceful life need medical and psychological assistance, retraining, employment, and full-fledged psychological and social rehabilitation [13 – 17].

Another global medical and social problem that humanity has faced in recent years is the pandemic of coronavirus infection caused by SARS-CoV-2 (COVID-19). Numerous studies have confirmed its negative impact not only on somatic, but also on mental health: patients who have had a Coronavirus infection have a higher prevalence of depressive and anxiety symptoms, as well as manifestations of post-traumatic stress disorder, which significantly exceeds the indicators inherent in the population as a whole [18 – 25].

At the same time, the impact of COVID-19 on the mental health of combatants has not been practically studied. The synergistic effect on the psyche of unfavorable factors of combat stress and coronavirus infec-

tion in such patients determines the specific nature of psychopathogenesis which affects the structure and features of psychopathological symptoms, and requires special approaches to treatment, rehabilitation and psychosocial adaptation of this contingent.

Based on the above, the study of the features of psychopathological symptoms in combatants who have suffered from COVID-19 is relevant, and can become the basis for improving modern approaches to the treatment of psychiatric consequences of military operations and coronavirus infection in these patients.

The research hypothesis was that combatants with non-psychotic mental disorders who recovered from COVID-19 have a worse affective state compared to combatants who did not have COVID-19.

THE AIM

The aim of the study was to establish the level of anxiety and depressive disorders and the impact of covid-19 on affective pathology in combatants with non-psychotic mental disorders who have suffered from COVID-19 in a comparative aspect.

MATERIALS AND METHODS

We clinically examined 252 male patients who were directly involved in combat operations, and who applied for psychiatric help in the Ternopil Regional Neuropsychiatric Hospital and for consulting and medical assistance in the Department of Psychiatry, Narcology and Medical Psychology of the Ternopil National Medical University named after I. Y. Gorbachevskiy during 2020-2022. All participants provided written informed consent, in accordance with all applicable regulatory and Good Clinical Practice guidelines and in full respect of the Ethical Principles for Medical Research Involving Human Subjects, as adopted by the 18th World Medical Association General Assembly (WMA GA), Helsinki, Finland (1964). The study received approval from the local ethics committee (Ethics Committee of the Ternopil National Medical University named after I. Y. Gorbachevskiy). The selection of patients for the study was carried out using a sampling method based on the following criteria: participation in hostilities and the establishment of a diagnosis of non-psychotic mental disorder to the criteria of ICD-10 (ICD – 11), the criterion for exclusion from the study was the presence of mental disorders of the psychotic register, narcological pathology, as well as concomitant severe somatic diseases. Among these patients, we identified two groups: patients who did not have COVID-19, numbering 132 people (group 1 – G1), and patients who had COVID-19, which was confirmed by enzyme immunoassay or polymerase chain reaction and recorded in the relevant medical documentation, numbering 120 people (group 2 – G2). Comparison of the results of clinical and psychopathological, psychodiagnostic and psychometric studies was carried out by comparing the indicators of patients of the first and second groups.

STUDY DESIGN

The study was carried out in two stages. At the first stage, patients were selected for the study and their medical records were studied. At the second stage, a clinical examination of patients and a psychometric study using scales of depression and anxiety were carried out. At the third stage, statistical processing, data analysis and generalization of the research results were made.

The study used clinical, anamnestic, psychometric and statistical methods. Clinical and anamnestic methods were used to assess the subjective manifestations of affective pathology and their dynamics, the psychometric method – for a standardized assessment of the level of depression and anxiety, the statistical method – to analyze the differences in indicators.

The examination included a clinical interview organized by the method of a semi-structured clinical interview, during which the presence and detail of complaints from the affective sphere was established, as well as an assessment of the severity of depressive and anxiety symptoms using the depression and anxiety scales of M. Hamilton (Hamilton Rating Scale for Depression – HDRS and Hamilton Anxiety Rating Scale – HARS) (M. Hamilton, 1959, 1960).

Statistical analysis of differences in categorized traits was performed using Fisher's exact test (one-sided), quantitative traits - using the nonparametric Mann-Whitney test.

RESULTS

The manifestations of affective pathology were the most common in the structure of psychopathological symptoms of the studied patients: complaints of anxiety or fear were found in 70.5±4.0% (confidence limits 66,5-74,5%) of patients of the G1, and 80.8±3.6% (77,2-84,4%) of patients of the G2 ($p<0.01$); low mood – in 55.3±4.4% (50,9-59,7%) and 66.7±4.3% (62,4-71,0%) ($p<0.05$); manifestations of emotional lability – in 44.7±5.0% (39,7-49,7%) and 58.3±4.5% (53,8-62,8%) ($p<0.05$); irritability – in 40.9±4.3% (36,6-45,2%) and 55.0±4.5% (50,5-59,5%) ($p<0.05$); emotional sensitivity – in 53.0±4.3% (48,7-57,3%) and 71.7±4.1% (67,6-75,8%) ($p<0.01$); dulling emotions – in 6.8±2.2% (4,6-9,0%) and 6.7±2.3% (4,4-9,0%) of patients ($p>0.05$).

When analyzing the prevalence of depressive symptoms to standardized criteria, it was found that among

Table I. HDRS indicators in combatants with non-psychotic mental disorders

Indicators	Value, points M±SD / Me (Q_{25}-Q_{75}) [conf. limits 95%]		p
	G1, n=132	G2, n=120	
Total score	15,29±4,16 / 15,00 (12,00–18,00) [14.57–16.00]	18,05±4,29 / 18,00 (15,00–21,00) [17.27–18.82]	<0,01
Adynamic depression	9,04±2,90 / 9,00 (7,00–11,00) [8.54–9.54]	10,58±2,71 / 10,00 (8,00–13,00) [10.09–11.07]	<0,01
Agitation depression	8,35±2,55 / 8,00 (7,00–10,00) [7.90–8.79]	9,63±2,61 / 9,00 (8,00–11,00) [9.16–10.10]	<0,01
Depression with fear	6,27±2,28 / 6,00 (5,00–8,00) [5.88–6.67]	7,26±2,05 / 7,00 (6,00–9,00) [6.88–7.63]	<0,01
Undifferentiated depression	5,60±1,80 / 5,00 (4,00–7,00) [5.29 – 5.91]	6,62±1,90 / 7,00 (5,00–8,00) [6.27–6.96]	<0,01

Table II. HARS indicators in combatants with non-psychotic mental disorders

Indicators	Value, points M±SD / Me (Q_{25}-Q_{75}) [conf. limits 95%]		p
	G1, n=132	G2, n=120	
Total score	20,52±7,14 / 21,00 (15,00–25,00) [19.28–21.74]	24,53±6,69 / 23,50 (21,00–28,50) [23.31–25.73]	<0,01
Mental anxiety	12,23±4,57 / 12,00 (9,00–15,50) [11.44–13.01]	14,74±3,71 / 14,00 (12,50–17,50) [14.07–15.41]	<0,01
Somatic anxiety	8,29±3,79 / 8,00 (6,00–11,00) [7.64–8.94]	9,78±3,94 / 9,00 (7,00–12,00) [9.07–10.49]	<0,01

the main symptoms of depression, the most common was anhedonia (loss of interest in previously pleasant and desirable activities, or loss of the ability to enjoy it): it was found in 77.3±3.7% (73.6-81.0%) of G1 patients, and in 83.3±3.4% (79.9-86.7%) of G2 patients (p>0.05), mood decline was also common – 55.3±4.4% (50,9-59,7%) and 66.7±4.3% (62,4-71,0%) (p<0.01), and rapid fatigue – 51.5±4.4% (47.1-55.9%) and 65.8±4.3% (61.5-70.1%) (p<0.05). Among the additional symptoms of depression to the standardized criteria, the leading place belonged to feelings of guilt, futility, anxiety or fear: these symptoms occurred in 78.0±3.6% (74.4-81.6%) of patients of the G1 and 87.5±3.0% (84.5-90.5%) of patients of the G2 (p<0.05), and manifestations of dissomnia: 47.0±4.3% (42.7-51.3%) and 61.7±4.4% (57.3-66.1%) (p<0.05); less common were the inability to concentrate and make decisions – 25.8±3.8% (22.0-29.6%) and 43.3±4.5% (38.8-47.8%) (p<0.01); thoughts of death or suicide – 25.0±3.8% (21.2-28.8%) and 35.8±4.4% (31.4-40.2%) (p<0.05); pessimism – 21.2±3.6% (17.6-24.8%) and 31.7±4.2% (27.5-35.9%) (p<0.05), low self-esteem – 21.2±3.6% (17.6-24.8%) and 31.7±4.2% (27.5-35.9%) (p<0.05), and the least common symptom is unstable appetite and weight loss – 17.4±3.3% (14.1-20.7%) and 24.2±3.9% (20.3-28.1%) (p>0.05). In general, the simultaneous presence of at least two main and three additional criteria for depression necessary for establishing a diagnosis of depressive disorder was found in 38 patients (28.8±3.9% (24.9-32.7%)) of the G1 and in 64 patients (53.3±4.6% (48.7-57.9%)) of the G2 (p<0.01).

Analysis of indicators of depressive symptoms using the M. Hamilton depression scale allowed us to quantify the severity of both individual components of affective disorders and depression in general in combatants who had and did not have COVID-19 (Table I).

A common trend identified in the study was the presence of significantly higher rates of depression severity in general and its individual clinical variants in combatants who had COVID-19. The overall rate of depression in combatants with non-psychotic mental disorders who did not have COVID-19 corresponded to moderate severity, closer to mild depressive disorder, and in patients who had COVID-19, moderate severity, was closer to severe depressive disorder. Combatants with COVID-19 had significantly higher rates of adynamic depression, agitated depression, fear depression, and undifferentiated depression.

Analysis of indicators for individual components of the M. Hamilton depression scale confirmed that the vast majority of depressive symptoms were more pronounced in combatants who had had COVID-19.

Thus, the indicator of depressive mood in patients of the G1 was 2.12±1.04 points, and in patients of the G2 – 2.48±1.05 points (p<0.01); guilt – 1.39±0.51 points and 1.53±0.52 points (p<0.05), suicidal intentions – 1.18±0.75 points and 1.35±0.83 points (p>0.05), which indicates a high level of severity of symptoms of depression, especially depressive mood in the examined patients.

The examined patients showed increased indicators of various forms of dissomnia: early insomnia – 0.53±0.77 points and 0.74±0.85 points (p<0.05); average insomnia – 0.49±0.75 points and 0.73±0.86 points (p<0.05), and late insomnia – 0.43±0.60 points and 0.69±0.76 points (p<0.01). The examined combatants were characterized by a pronounced increase in indicators of disability and

activity – 2.56±1.01 points and 2.85±0.90 points ($p<0.05$), and lethargy – 1.36±0.66 points and 1.44±0.58 points, and agitation – 0.77±0.88 points and 0.82±0.93 points, although the latter discrepancies are not statistically significant ($p>0.05$).

In addition to depressive anxiety, the examined patients were found to have increased levels of anxiety, more pronounced in patients who had had COVID-19: mental anxiety – 2.87±1.59 points and 3.28±1.30 points ($p<0.05$), and somatic anxiety – 1.53±0.90 points and 1.82±0.97 points ($p<0.05$).

A significant place among the manifestations of depression in the studied patients is occupied by somatic symptoms of depressive disorders: gastrointestinal symptoms – 0.55±0.56 points and 0.52±0.52 points ($p>0.05$); general somatic symptoms – 0.97±0.65 points and 1.28±0.75 points ($p<0.01$) and genital symptoms – 1.02±0.68 points and 1.07±0.64 points ($p>0.05$).

Hypochondria indicators were also increased – 0.71±0.90 points and 1.04±0.95 points, and discrepancies between the groups were statistically significant ($p<0.01$). Weight loss rates in the studied patients were low – 0.22±0.51 points and 0.31±0.56 points ($p>0.05$). Combatants showed a high level of criticality for their condition, slightly worse in patients who had suffered COVID-19 – 0.00±0.00 points and 0.03±0.16 points ($p>0.05$).

The examined combatants showed low and significantly indistinguishable indicators of daily mood swings – 0.10±0.30 points and 0.08±0.28 points ($p>0.05$), depersonalization and derealization – 0.39±0.94 points and 0.36±0.86 points ($p>0.05$); paranoid symptoms – 0.00±0.00 points and 0.00±0.00 points ($p>0.05$).

The examined patients found a high level of obsessive and compulsive symptoms, significantly higher in patients who had had COVID-19 – 0.97±1.07 points and 1.28±1.19 points ($p<0.05$).

In addition to depressive symptoms, in the structure of affective manifestations of non-psychotic mental disorders in combatants, an important place is occupied by manifestations of anxiety which were detected in most of the examined patients. Quantitative assessment of the severity of anxiety phenomena using the M. Hamilton anxiety scale allowed us to give a detailed description of the manifestations of anxiety in the studied contingent.

In general, the combatants surveyed had increased levels of anxiety (Table II).

The overall anxiety score of combatants with non-psychotic mental disorders corresponded to the average level, notably the patients without COVID-19 had this indicator approaching the low level, and patients who had COVID-19 it was close to the high level limit. In the structure of anxiety, elevated levels were also found for its individual types: mental and somatic anxiety.

The examined combatants were found to have increased values for certain indicators of the M. Hamilton anxiety scale. Thus, the indicator of anxiety mood in combatants who did not have COVID-19 was 2.87±1.59 points, and in combatants who had COVID-19, it was 3.28±1.30 points ($p<0.05$); tension – 2.51±1.21 points and 2.78±1.26 points ($p<0.05$); fears – 1.76±1.16 points and 1.90±0.91 points ($p>0.05$).

In the structure of anxiety symptoms, increased indicators of insomnia were found – 1.41±1.48 points and 2.03±1.60 points ($p<0.01$), cognitive disorders – 1.56±1.06 points and 2.27±0.77 points ($p<0.01$), as well as depressive mood – 2.12±1.04 points and 2.48±1.05 points ($p<0.01$), which is consistent with the indicators obtained using the M. Hamilton depression scale.

The studied patients showed increased indicators of somatic muscle symptoms (pain, muscle twitching, tension, cramps, teeth grinding, breaking voice, increased muscle tone) – 0.98±1.10 points and 1.20±1.07 points ($p>0.05$) and somatic sensory symptoms (ringing in the ears, blurred vision, hot or cold flashes, feeling weak, tingling) – 0.80±1.07 points and 1.11±1.18 points ($p<0.05$).

Similarly, a study using the M. Hamilton anxiety scale confirmed a high level of somatic symptoms of affective disorders in the studied patients: cardiovascular symptoms (tachycardia, palpitations, chest pain, pulsation in blood vessels) – 1.16±1.07 points and 1.53±1.07 points ($p<0.05$); respiratory symptoms (feeling of pressure and compression in the chest, shortness of breath, rapid breathing) – 0.60±0.91 points and 0.88±1.03 points ($p<0.05$); gastrointestinal symptoms (difficulty swallowing, flatulence, abdominal pain, heartburn, feeling full stomach, nausea, vomiting, abdominal rumbling, diarrhea, constipation, weight loss) – 1.02±0.68 points and 1.07±0.64 points ($p>0.05$). At the same time, with higher values of indicators in patients who had had COVID-19, discrepancies between the groups were statistically significant only for cardiovascular symptoms, and not significant for respiratory and gastrointestinal symptoms. Elevated indicators were also found for genitourinary symptoms (frequent urination, strong urge to urinate, decreased sexual desire, erectile dysfunction) – 1.02±0.68 points and 1.07±0.64 points ($p<0.05$) and vegetative symptoms (dry mouth, redness or pallor of the skin, sweating, headaches, feeling of tension) – 1.08±1.37 points and 1.31±1.40 points ($p>0.05$), as well as for examination behavior – 1.64±1.13 points and 1.63±1.10 points ($p>0.05$).

DISCUSSION

Our data on the significant prevalence of affective pathology in combatants are consistent with a number of recent

studies that have identified pronounced manifestations of depression, anger, anxiety, shame and guilt underlying the pathological affective response in combatants [26, 27].

It should be noted that despite the pre-nosological differences in the personal and motivational characteristics of combatants, which play an important role as factors of predisposition of affective pathology, there are certain general patterns in the manifestations of affective response of persons exposed to combat stress, consisting primarily in the core nature of depressive and anxiety symptoms in psychopathological constructs in this category of patients, which is confirmed in our study [28, 29].

At the same time, the polymorphism of depressive and anxiety symptoms, the significant prevalence of somatovegetative and dissomnic manifestations in combatants identified in our study indicate the complex polyfactorial nature of changes in the affective sphere and the need for differentiated approaches to providing such patients with psychiatric and rehabilitation care, which is emphasized by other authors [30].

It should be noted that the combatants we studied who suffered from COVID-19 showed more significant manifestations of affective symptoms, in particular, depression and anxiety, compared to combatants who did not have a coronavirus infection. These data correspond to the results of studies of other authors, who provide data on the prevalence of depressive symptoms in 14% - 48% of patients with COVID-19, and anxiety – in 6% - 51% of patients [18-24].

At the same time, the quantitative severity and prevalence of depressive and anxiety phenomena in combatants identified in our study was greater than that found in similar studies on the civilian population (depression – 66.7%, anxiety – 80.8%), which allows us to consider the factors of participation in hostilities and the disease COVID-19 as mutually aggravating factors in relation to the risk of affective pathology, in particular, depressive and anxiety disorders.

CONCLUSIONS

1. Combatants with non-psychotic mental disorders are characterized by high levels of affective pathology, in particular, depressive and anxiety disorders: 28.8±3.9% (24.9-32.7%) in combatants who did not have COVID-19 and 53.3±4.6% (48.7-57.9%) in combatants who had COVID-19 ($p<0.01$).
2. COVID-19 disease aggravates the course of affective pathology in combatants with non-psychotic mental disorders and increases the incidence of their depressive and anxiety symptoms: anxiety or fear (70.5±4.0% (66,5-74,5%) and 80.8±3.6% (77,2-84,4%), $p<0.01$ respectively), low mood (55.3±4.4% (50,9-59,7%) and 66.7±4.3% (62,4-71,0%), $p<0.05$ respectively); emotional lability (44.7±5.0% (39,7-49,7%) and 58.3±4.5% (53,8-62,8%), $p<0.05$ respectively); irritability (40.9±4.3% (36,6-45,2%) and 55.0±4.5% (50,5-59,5%), $p<0.05$ respectively; emotional sensitivity (53.0±4.3% (48,7-57,3%) and 71.7±4.1% (67,6-75,8%), $p<0.01$ respectively; dulling emotions (6.8±2.2% (4,6-9,0%) and 6.7±2.3% (4,4-9,0%), $p>0.05$ respectively).
3. The quantitative indicators of the severity of depression in combatants correspond to the moderate severity of depressive disorder; at the same time, in combatants who did not have COVID-19, the indicators are closer to mild depressive disorder (15,29±4,16 / 15,00 (12,00–18,00) [14.57–16.00] points), and in combatants who had COVID-19 – to severe depressive disorder (18,05±4,29 / 18,00 (15,00–21,00) [17.27–18.82] points, $p<0,01$). Combatants who suffered from COVID-19 had significantly higher rates of certain types of depression: adynamic (9,04±2,90 / 9,00 (7,00–11,00) [8.54–9.54] points and 10,58±2,71 / 10,00 (8,00–13,00) [10.09–11.07] points, $p<0,01$ respectively), agitated (8,35±2,55 / 8,00 (7,00–10,00) [7.90–8.79] points and 9,63±2,61 / 9,00 (8,00–11,00) [9.16–10.10] points, $p<0,01$ respectively), fearful (6,27±2,28 / 6,00 (5,00–8,00) [5.88–6.67] points and 7,26±2,05 / 7,00 (6,00–9,00) [6.88–7.63] points, $p<0,01$ respectively), and undifferentiated (5,60±1,80 / 5,00 (4,00–7,00) [5.29 – 5.91] points and 6,62±1,90 / 7,00 (5,00–8,00) [6.27–6.96] points, $p<0,01$ respectively) one.
4. The overall anxiety index of combatants with non-psychotic mental disorders corresponded to the average level, while in patients who did not have COVID-19, the indicator was closer to the low level (20,52±7,14 / 21,00 (15,00–25,00) [19.28–21.74] points), and in patients who had COVID-19 – close to the high level limit (24,53±6,69 / 23,50 (21,00–28,50) [23.31–25.73] points, $p<0,01$). In the structure of anxiety, elevated levels were also found for its individual types: mental (12,23±4,57 / 12,00 (9,00–15,50) [11.44–13.01] points and 14,74±3,71 / 14,00 (12,50–17,50) [14.07–15.41] points, $p<0,01$ respectively) and somatic (8,29±3,79 / 8,00 (6,00–11,00) [7.64–8.94] points and 9,78±3,94 / 9,00 (7,00–12,00) [9.07–10.49] points, $p<0,01$ respectively) anxiety.
5. The identified patterns should be taken into account during the development of treatment and rehabilitation measures for combatants with non-psychotic mental disorders who have suffered from COVID-19.

Prospects for further research are related to the study of the full range of psychopathological symptoms in combatants who have suffered from COVID-19, in particular, cognitive disorders, as well as to the improvement of modern approaches to the treatment and rehabilitation of this category of patients based on the data obtained.

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ORIGINAL ARTICLE

IMPACT OF CLINICAL CHARACTERISTICS OF LYME BORRELIOSIS ON PATIENTS' LIFE QUALITY

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ABSTRACT

The aim: To study the impact of clinical characteristics of the disease on the life quality of patients with Lyme borreliosis.

Materials and methods: Forty-eight (33 women and 15 men) patients with Lyme borreliosis aged 23-77 years and 48 individuals who did not have any somatic diseases that would impact the quality of life (experimental group) were examined. The MOS 36-item Short Form Health Survey (SF-36) was used to study the quality of life. Cognitive function was assessed using the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA).

Results: According to the SF-36 questionnaire, patients with Lyme borreliosis had a significant decrease in scores for all indicators of physical health ($p < 0.001$), as well as impaired social ($p < 0.001$) and emotional ($p = 0.027$) functioning compared with healthy individuals. The deterioration of the quality of life indicators essentially depends on the clinical characteristics of the disease, namely its duration, the presence of Lyme arthritis, neuroborreliosis, cognitive disorders, multiple organ involvement, disease stage.

Conclusions: Lyme borreliosis and its certain clinical characteristics have a significant impact on the life quality of patients.

KEY WORDS: Lyme borreliosis, quality of life, infectious

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INTRODUCTION

Lyme borreliosis (tick-borne borreliosis) is an infectious focal disease caused by several Borrelia species pathogenic for humans (B. burgdorferi, B. Garinii, B. Afzelii) and transmitted by the bite of infected ticks. The first disease manifestations might be a characteristic erythema at the site of the tick bite and/or flu-like symptoms, such as fever, fatigue, general feeling of being unwell [1]. At the same time, Lyme borreliosis is not diagnosed in time in a significant number of patients, because the carrier tick is small, its bite may be painless, and the characteristic erythema may be absent [1]. Late diagnosis, lack of treatment or a too short course of antibiotic therapy can lead to multisystem lesions with significant polymorphism of clinical manifestations or to the development of latent forms with a high risk of chronicity [2-4]. There is also evidence that 36% of patients treated at an early stage of Lyme borreliosis remain ill after the treatment [5].

Lyme borreliosis can be associated with severe pain and functional disorders, on the one hand, and psycho-emotional disorders, on the other hand, which can lead to disability and a significantly reduced quality of life of patients. Thus, there are a number of factors, such as clinical, social, and probably psychological, that can affect the well-being of patients with Lyme disease. At the same time, very little data is available on the impact of this disease and, in particular, its certain clinical characteristics, on the life quality of patients.

A study of the quality of life with Lyme borreliosis will help analyze patients' physical and mental status, their attitude towards the disease, as well as evaluate the effectiveness of the therapy and the course of the disease [6-8].

The main hypothesis of our study was the assumption that the clinical characteristics of Lyme borreliosis significantly affect the quality of life.

Identification of modified predictors of the formation of negative dynamics of quality of life will help to stratify patients for more thorough dynamic control for timely correction of therapy.

THE AIM

The aim was to study the impact of clinical characteristics of the disease on the life quality of patients with Lyme borreliosis.

MATERIALS AND METHODS

A prospective case-control study was carried out at the Center of Infectious Disorders of the Nervous System of the State Institution "Hromashevskyi Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine" from January 1, 2014 to November 30, 2022.

By analyzing medical records of inpatients (form 003/o) and medical records of outpatients (form No. 025/o), collecting subjective and objective anamnestic data, conducting a standard neurological examination, the case group was formed, which included 48 (33 women and 15 men) patients with a confirmed diagnosis of Lyme borreliosis aged 23-77 years (mean age (M±σ) – 49.4±16.02 years). The comparison group consisted of 48 people matched for gender, age, nationality, and place of birth, who did not have any somatic diseases that would impact the quality of life. The results of a questionnaire survey of healthy individuals, students, and clinic employees were used.

The inclusion criteria were as follows: 1) age from 18 years; 2) confirmed diagnosis of Lyme borreliosis by a two-stage method with the study of blood serum for IgM and IgG antibodies to Borrelia by ELISA and immunoblotting, as well as cerebrospinal fluid for specific antibodies to Borrelia; 3) patient informed consent.

The exclusion criteria included the following: age < 18 years; decompensated concomitant somatic, oncological, mental, neurological condition; dementia of different genesis; incompletely filled-in questionnaire; refusal to participate in the study.

All patients underwent an assessment of cognitive status using the Mini-Mental State Examination (MMSE) [9] and the Montreal Cognitive Assessment (MoCA) [10].

The MOS 36-item Short Form Health Survey (SF-36) was used to assess the quality of life [11]. It consists of 36 questions on 8 main aspects of health, namely: physical functioning (PF), role physical (RP), role emotional (RE), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), and mental health (MH).

Statistical processing of data was performed in statistical package "SPSS 13" (IBM, the USA), owned by the Kyiv Center of Political Science and Conflict Studies, license number CA23452175787B.

Quantitative indicators were tested to follow a normal distribution using the

Shapiro-Wilk test and presented as M±σ (mean value ± standard deviation). A two-tailed Students t-test (for two independent samples) was used to compare quantitative data.

Qualitative indicators are presented in absolute and relative values (%). To find differences between quality indicators, the Pearson's chi-square (χ^2) test was used with Yates' correction for continuity, for the calculation of which a 2x2 grid was built.

The correlation between the studied indicators was evaluated based on the results of correlation analysis with the calculation of the Spearman's correlation coefficient (R) and the subsequent determination of its significance by the t-criterion. P<0.05 (95% confidence interval) was considered as the acceptable significance level.

RESULTS

The study involved 48 patients with Lyme borreliosis aged 23-77 years (mean age (M±σ) – 49.4±16.02 years) and the disease duration 3.02±3.72 years on average.

The main clinical characteristics of the examined patients with Lyme borreliosis who were included in the study are presented in Table I.

According to clinical and pathogenetic classification offered by Yu.V. Lobzin (1996), 50% of patients with Lyme borreliosis included in the study had an early stage of Lyme borreliosis, and 50% of them had an advanced stage. Twenty (41.7%) patients were diagnosed with neuroborreliosis, 7 (14.6%) patients had a predominance of the symptoms of cardiovascular system damages in the clinical picture of the disease, 9 (18.7%) patients had joint damages (Lyme arthritis), and 1 (2.1%) patient had eye disorders. Eleven (22.9%) patients were diagnosed with neuroborreliosis associated with multiple organ involvement.

According to MMSE, the average score of cognitive status in patients with Lyme borreliosis was 26.89 ± 2.46 scores, while in healthy individuals it was 27.97 ± 1.82 scores (p=0.016). According to MoCA, it was 24.6±2.73 and 26.71±1.58 scores respectively (p<0.001). Thirty-one patients with Lyme borreliosis had cognitive disorder according to MMSE <28 scores and MoCA <26, representing 64.6% of the total.

Quality of life indicators were significantly lower in patients with Lyme borreliosis: PF by 35.5% (51.25 ± 23.35% vs 79.48 ± 16.54% in healthy individuals, p<0.001), RP by 33.6% (51.56 ± 30.69% vs 77.6 ± 18.04% respectively, p<0.001), BP by 29.3% (48.06 ± 14.64% vs 68.02 ± 16.85%, p<0.001), GH by 34.6% (23.54 ± 8.75% vs 35.98 ± 13.13%, p<0.001), SF by 2.7 times (25.26 ± 14.7% vs 68.63 ± 20.04%, p<0.001), and RE by 32.3% (28.19 ± 28.36% vs 41.64 ± 30.37%, p=0.027) (Table II).

A correlation analysis was carried out to determine the degree of influence of clinical characteristics of Lyme borreliosis on the quality of life indicators according to the SF-36 questionnaire (Table III).

An inverse correlation between the disease duration and a decrease in quality of life indicators such as PF (R =-0.371, p = 0.01), RP (R =-0.392, p = 0.006), BP (R =-0.425, p = 0.003) and SF (R =-0.297, p = 0.04) was established.

Table I. Main clinical characteristics of patients (n=48) with Lyme borreliosis (M ± σ)

Indicator	Indicator values
Age – years, M ± σ (range)	49.4 ± 16.03 (23 – 77)
Disease duration – years, M ± σ (range)	3.02 ± 3.72 (0.16 – 20)
Clinical manifestations of the disease, n (%):	
neuroborreliosis	20 (41.7%)
cardiovascular system damages	7 (14.6%)
eye disorders	1 (2.1%)
Lyme arthritis	9 (18.7%)
neuroborreliosis and multiple organ involvement	11 (22.9%)
Disease stage, n (%):	
early disseminated	24 (50%)
advanced	24 (50%)
MMSE – scores, M ± σ (range)	26.89 ± 2.46 (20 – 30)
MoCA – scores, M ± σ (range)	24.6 ± 2.73 (18 – 30)

Notes: Arithmetical mean values of parameters studied (M) and standard deviations (σ) are presented in the table.

Table II. Quality of life indicators in patients with Lyme borreliosis (n=48) and healthy individuals of the experimental group (n=48)

Quality of life indicators	Patients with Lyme borreliosis (n=48)	Healthy individuals (n=48)
	M ± σ	M ± σ
Physical functioning (PF)	51.25 ± 23.35*	79.48 ± 16.54
Role physical (RP)	51.56 ± 30.69*	77.6 ± 18.04
Bodily pain (BP)	48.06 ± 14.64*	68.02 ± 16.85
General health (GH)	23.54 ± 8.57*	35.98 ± 13.13
Vitality (VT)	32.81 ± 13.16	34.9 ± 17.02
Social functioning (SF)	25.26 ± 14.7*	68.63 ± 20.04
Role emotional (RE)	28.19 ± 28.36*	41.64 ± 30.37
Mental health (MH)	30.25 ± 12.46	34.42 ± 12.98

Notes:
1. Arithmetical mean values of parameters studied (M) and standard deviations (σ) are presented in the table;
2. * – statistically significant difference ($p < 0.05$) compared to values in the control group.

Neuroborreliosis, cognitive impairment, joint damages (Lyme arthritis), multiple organ involvement, and advanced stage of the disease were also correlated with lower scores on patient physical health components such as PF ($R = -0.356$, $p = 0.013$; $R = -0.610$, $p < 0.001$; $R = -0.351$, $p = 0.014$; $R = -0.426$, $p = 0.003$; $R = -0.343$, $p = 0.017$ respectively, RP ($R = -0.428$, $p = 0.002$; $R = -0.500$, $p < 0.001$; $R = -0.395$, $p = 0.005$; $R = -0.518$, $p < 0.001$; $R = -0.497$, $p < 0.001$ respectively), and BP ($R = -0.409$, $p = 0.004$; $R = -0.367$, $p = 0.01$; $R = -0.286$, $p = 0.048$; $R = -0.252$, $p = 0.084$; $R = -0.634$, $p < 0.001$ respectively).

In addition, neuroborreliosis, cognitive impairment, multiple organ involvement, and advanced stage of the disease were reliably correlated with the deterioration of mental health components, such as VT ($R = -0.475$, $p = 0.001$; $R = -0.492$, $p < 0.001$; $R = -0.365$, $p = 0.011$; $R = -0.408$, $p = 0.004$ respectively) and RE $R = -0.550$, $p < 0.001$; $R = -0.757$, $p < 0.001$; $R = -0.312$, $p = 0.031$; $R = -0.431$, $p = 0.002$ respectively).

The SF indicator changed considerably in case of neuroborreliosis ($R = -0.361$, $p = 0.012$), cognitive disorder ($R = -0.286$, $p = 0.04$), joint damages (Lyme arthritis) ($R = -0.319$, $p = 0.027$), advanced stages of the disease ($R = -0.412$, $p = 0.004$), and the presence of cognitive disorders had a reliable correlation with the physical component of life quality ($R = -0.328$, $p = 0.023$).

DISCUSSION

Lyme borreliosis is a multisystem disease that can affect the skin, joints, heart, eyes, and nervous system [12]. A significant number of patients are not diagnosed with Lyme borreliosis in time [1]. Late diagnosis, lack of treatment, or a too short course of antibiotic therapy can lead to multisystem lesions with significant polymorphism of clinical manifestations or to the development of latent forms with a high risk of chronicity [2-4]. There is evidence

Table III. Correlations of quality of life indicators in patients with Lyme borreliosis (according to the SF-36 questionnaire) with clinical characteristics of the disease

Quality of life indicators	Disease duration	Neuroborreliosis	Lyme arthritis	Damage to several organs	Advanced stage of the disease	Cognitive disorders
Physical functioning (PF)	R =-0.371* p = 0.01	R =-0.356* p = 0.013	R =-0.351* p = 0.014	R =-0.426** p = 0.003	R =-0.343* p = 0.017	R =-0.610** p < 0.001
Role physical (RP)	R =-0.392** p = 0.006	R =-0.428** p = 0.002	R =-0.395** p = 0.005	R =-0.518** p = 0.000	R =-0.497** p = 0.000	R =-0.500** p < 0.001
Bodily pain (BP)	R =-0.425** p = 0.003	R =-0.409** p = 0.004	R =-0.286* p = 0.048	R =-0.252 p = 0.084	R =-0.634** p = 0.000	R =-0.367* p = 0.01
General health (GH)	R =-0.132 p = 0.371	R =0.256 p = 0.079	R =0.199 p = 0.176	R =-0.199 p = 0.176	R =-0.246 p = 0.092	R =-0.127 p = 0.388
Vitality (VT)	R =-0.224 p = 0.126	R =-0.475** p = 0.001	R =0.023 p = 0.879	R =-0.365* p = 0.011	R =-0.408** p = 0.004	R =-0.492** p < 0.001
Social functioning (SF)	R =-0.297* p = 0.04	R =-0.361* p = 0.012	R =-0.319* p = 0.027	R =-0.138 p = 0.351	R =-0.412** p = 0.004	R =-0.286* p = 0.04
Role emotional (RE)	R =-0.133 p = 0.367	R =-0.550** p = 0.000	R =0.195 p = 0.185	R =-0.312* p = 0.031	R =-0.431** p = 0.002	R =-0.757** p < 0.001
Mental health (MH)	R =0.027 p = 0.857	R =0.204 p = 0.164	R =-0.138 p = 0.351	R =-0.003 p = 0.984	R =-0.264 p = 0.070	R =-0.328 p = 0.023

Notes:
1. * – statistically significant difference ($p < 0.05$);
2. ** – statistically significant difference ($p < 0.01$).

from several studies that such patients tend to report chronic pain, fatigue, and cognitive disorders, even though they have received standard courses of antibiotic therapy [13, 14]. These clinical signs and symptoms certainly have an impact on the quality of life of patients.

The obtained results, as well as the data of other authors, show that Lyme borreliosis has a significant impact on the quality of life of patients [7, 8]. It was found that, patients with tick-borne borreliosis had a significant decrease in scores for all indicators of physical health, as well as impaired social and emotional functioning according to the SF-36 questionnaire compared with healthy individuals. The latter indicates the negative impact of the disease not only on the physical, but also on the mental health of patients.

The results of the study show that the quality of life of patients with Lyme borreliosis worsens as the duration of the disease increases, which might be related to the chronicity of the process, partial or complete disability, and age-related comorbid conditions and pathologies in the patient population. In particular, according to L.R. Johnson et al. (2014), about 22.8% of respondents reported disability due to Lyme disease and the need for special medical equipment (cane, crutches, wheelchair) [7]. Klempner et al. (2001) found that patients with Lyme disease can have a degree of disability equivalent to that of patients with congestive heart failure [15].

Chronic pain and loss of physical function are the most serious consequences of the inflammatory process in the joints and have a great impact on various spheres of human life [16, 17]. This explains the obtained correlations between Lyme arthritis and a decrease in scores on patient physical health components such as PF, RP, and BP, as well as mental health – SF. Since the disease progression is unpredictable, patients begin to worry about the future, the increasing limitation of mobility, the risk of inability to self-care, which has a negative impact on all spheres of their life [18].

Approximately 75% of patients with Lyme borreliosis report severe or very severe symptoms associated with pain in the joints or in other parts of the body, cognitive disorders, sleep disturbance, or mood change, and 63% have more than one severe or very severe symptom [7].

The study focuses on the fact that neuroborreliosis, cognitive disorders, multiple organ involvement, and advanced stage of the disease contribute greatly to impaired quality of life, both physical and mental, in patients with Lyme borreliosis. Cognitive disorders play a significant role in decreasing mental functioning. Regarding the latter it should be noted that SF-36 is not a sufficient or sensitive instrument for detecting functional disorders in the cognitive sphere [19].

CONCLUSIONS

1. It was found that Lyme borreliosis has a significant impact on the life quality of patients compared to healthy individuals. According to the SF-36 question-

naire, the Physical Functioning indicator decreased by 35.5%, Physical Role Functioning – by 33.6%, Bodily Pain – by 29.3%, General Health – by 34.6%, Social Functioning – by 2.7 times ($p<0.001$), and Emotional Role Functioning – by 32.3% ($p = 0.027$).

2. The relationship between the negative dynamics of indicators of the physical components of health (Physical Functioning, Physical Role Functioning, Bodily Pain) and the presence of neuroborreliosis, cognitive disorders, Lyme arthritis, multiple organ involvement, as well as an increase in the duration of the disease ($p<0.05$) was established. On the other hand, the deterioration of mental health components (Vitality, Social Functioning, Emotional Role Functioning) was associated with the presence of neuroborreliosis, cognitive impairment, and the late stage of the disease ($p<0.05$).

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ORIGINAL ARTICLE

RISK FACTORS FOR GC-RESISTANT PULMONARY SARCOIDOSIS

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ABSTRACT

The aim: The study aimed to conduct a retrospective analysis of unfavorable outcome rate and to search for clinical and anamnestic criteria for predicting glucocorticoid-resistant pulmonary sarcoidosis.

Materials and methods: There were examined 37 women and 31 men with stage II to III pulmonary sarcoidosis from 2018-2022. The mean patients' age was (35.7±6.6) years. All patients underwent a chest computed tomography scan on the Toshiba Aquilion Prime CT scanner before the start of treatment and after the three-month glucocorticoid therapy. Anamnestic, age- and gender related factors of unfavorable treatment outcomes were studied.

Results: Dyspnea (86%), coughing (67%), general weakness and fatigue (29%) on the background of maintaining the indicators of lung tissue density at the level of -893.5 Hounsfield units and above according to the chest computed tomography imaging represented the three-month treatment failure. Glucocorticoid-resistant sarcoidosis was most diagnosed in patients with stage III disease; the mean patients' age was (44.3±3.2) years; B positive men prevailed; 85% of patients developed extrapulmonary manifestations; in 43% of cases, concomitant cardiovascular pathology was diagnosed.

Conclusions: Age, gender, comorbid conditions, extrapulmonary lesions, and blood type can be used as predictive criteria for GC-resistant sarcoidosis.

KEY WORDS: pulmonary sarcoidosis, diagnosis, glucocorticoid resistance

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INTRODUCTION

Interstitial lung diseases (ILDs) are increasingly becoming a very real challenge for modern medicine and continue to be the research focus of many scientists due to the continuous increase in their morbidity and mortality rates, the difficulties in the diagnosis and treatment [1-3].

To date, sarcoidosis is one of the most common ILDs of unknown etiology. In Belgium, this pathology accounts for 27% of all ILD cases, in Greece – 34.1%, in Italy – 33.7% [1-2].

Predicting the clinical course of pulmonary sarcoidosis poses a real challenge for clinicians as, according to different authors, its relapse rate after a discontinuation of anti-inflammatory agents ranges from 13% to 74% depending on the population. Relapse episodes are usually observed 1-12 months after a discontinuation of glucocorticoids (GCs) or their dose reduction. Patients with a remission for five months show the lowest risk of relapse episodes [4-6].

Despite the development of radiological and laboratory diagnostic methods, the search for the reliable clinical and anamnestic criteria that would provide for predicting the clinical course of the disease and assessing treatment efficacy are extremely relevant.

A wide range of aspects concerning the formation, development, and progression of pulmonary sarcoidosis remain poorly understood and debatable. Thus, a targeted study of sarcoidosis structure allows for deepening our understanding of etiological and pathogenetic factors affecting disease development, as well as for designing an individual prognosis for each patient, selecting an adequate therapeutic strategy, and assessing treatment efficacy [1, 4, 7].

It is worth mentioning that the treatment of sarcoidosis patients has been discussed ever since sarcoidosis was defined as a separate illness. Since 1999, GCs have remained the gold standard for sarcoidosis treatment, although there is still no consensus on when, in what cases, and at what doses GC therapy should be started [1-2].

Symptom severity, clinical course, and therapeutic strategy in sarcoidosis directly depend on the localization of the process, the presence of extrapulmonary manifestations, and the degree of inflammatory process activity [3, 5, 8].

THE AIM

The study aimed to conduct a retrospective evaluation of symptoms in patients with failure of treatment of pulmonary sarcoidosis after a 3-month course of steroid therapy and to develop prognostic criteria for the development of GC-resistant pulmonary sarcoidosis.

MATERIALS AND METHODS

Prior to the therapy and after the three-month treatment, an in-depth instrumental examination of 68 patients with pulmonary sarcoidosis was carried out. The mean patients' age was (35.7±6.6) years. Medical documentation was studied and 68 patients were examined in the period from 2018 to 2022 (extracts from outpatient and inpatient charts of patients).

All patients underwent general physical examination and some clinical laboratory tests, including a chest computed tomography (CT) scan, were made. Chest CT was conducted on the Toshiba Aquilion Prime CT scanner, with the scan results recorded on a digital medium and lung tissue density assessed in Hounsfield units (HU): if lung tissue density was less than -893.5 HU, a treatment was considered as effective, if lung tissue density exceeded -893.5 HU, a treatment was regarded as ineffective.

To find the possible causes of chronicity and progression of the process in pulmonary sarcoidosis, a detailed analysis of all the cases with unfavorable treatment outcomes, considering patients' age and gender, disease stage, symptom severity, extrapulmonary manifestations, and comorbidities, was conducted.

Methods of statistical analysis of research results. For the objectivity of judging the reliability of the research results, we used the variational statistical method of analyzing the obtained results with the help of a personal computer and an application program for working with Microsoft Office Excel 2010 spreadsheets using the Student's t-test, correlation and variance analysis using the "STATISTICA 6.1" package ("StatSoftInc", serial number AGAR909E-415822FA). During statistical processing, the arithmetic mean value (M), the probability of the difference in the research results (P). The results were considered probable when the reliability coefficient was less than or equal to 0.05, which is generally accepted in medical and biological research.

GC therapy included methylprednisolone at a dose of 0.4 mg/kg/day for four weeks, followed by a gradual dose reduction to 0.2 mg/kg/day until the end of the third month.

After the three-month treatment, based on patients' complaints, physical examination data, and chest CT scan results, all the patients were divided into two groups:

- Group I – treatment success – included 47 patients with positive clinical and radiologic outcomes;
- Group II – treatment failure – comprised 21 patients with persistent and/or increasing symptoms and no positive radiographic findings.

RESULTS

The results of studying clinical symptoms and instrumental data in patients with stage II to III active pulmonary sarcoidosis were analyzed before the start of treatment.

Coughing was the most common subjective symptom: 37 (71.15%) patients experienced a dry cough; in 15 (28.85%) cases, coughing was accompanied by sputum production; 16 (23.53%) patients reported no cough. Dyspnea was typical for studied patients as well; it was observed in 40 (58.82%) subjects. Excessive exercise-induced shortness of breath was seen in 26 (65%) cases; 13 (32.5%) patients experienced shortness of breath during moderate physical activity and 1 (2.5%) patient felt short of breath during little physical activity.

General weakness and fatigue, which were present in 28 (41.17%) subjects, were common symptoms as well.

Chest pain and discomfort were the least common – in 6 (8.82%) cases.

During the treatment, we observed significant positive changes in patients of Group I. All the patients of this group (n=47) demonstrated normalization/improvement of their general condition, reduction in/absence of coughing, dyspnea, chest pain.

On the background of the three-month treatment in patients of Group II, clinical changes were insignificant: cough frequency reduced in 7 (33.33%) patients and the intensity of dyspnea changed positively in 4 (14.29%) cases only. Persistent/progressive dyspnea was found in 17 (85.71%) individuals. A total of 14 (66.67%) patients developed coughing or had episodes of intense coughing. Chest pain persisted in one (4.76%) case. Six (28.57%) patients continued to feel general weakness and fatigue.

At the beginning of the study, a detailed analysis of all the patients by age, gender, radiologic disease stage, extrapulmonary manifestations, and comorbidities, was conducted.

The study included 31 women and 37 men; the mean patients' age was (35.7±6.6) years (Table I).

Stage II pulmonary sarcoidosis prevailed – 58 (85.29%) cases.

The distribution of patients by the radiologic stage is presented in Table I.

The most common comorbidities were cardiovascular and gastrointestinal diseases – in 19 (27.94%) and 11 (16.17%) cases, respectively (Table II). Urinary system disorders, ear, nose and throat (ENT) pathology, and

Table I. Distribution of patients depending on the radiologic stage of the disease

Stage	Women		Men	
	abs.	%	abs.	%
II	31	45.6±6,0	27	39.7±6,0
III	6	8.8±3,0	4	5.9±3,0

Table II. Frequency of comorbidities in patients with active pulmonary sarcoidosis

Comorbidity	Detection rate (number of cases)	
	abs.	%
Cardiovascular pathology	19	27.9±6,0
Respiratory pathology	5	7.4±3,0
Ophthalmic pathology	8	11.8±4,0
Gastrointestinal pathology	11	16.2±5,0
ENT pathology	3	4.4±3,0
Urinary system pathology	2	2.9±2,0
Anemia	1	1.8±2,0
Endocrine pathology	4	5.9±3,0

Table III. Frequency of extrapulmonary lesions in patients with sarcoidosis (Ivano-Frankivsk region)

Localization	Detection rate (number of cases)	
	abs.	%
Peripheral lymph nodes	8	11.8±4,0
Spleen	2	2.9±2,0
Eyes	1	1.5±2,0
Skin	1	1.5±2,0
Liver	1	1.5±2,0

anemia were the least common – in 2 (2.94%), 3 (4.41%), and 1 (1.47%) cases, respectively.

Sixteen (23.52%) patients were diagnosed with obesity of varying degrees.

Most patients, 61 subjects (89.71%), both men and women, have never smoked.

In all the 68 patients, the frequency of extrapulmonary manifestations of pulmonary sarcoidosis was analyzed in detail (Table III).

There were 13 cases of extrathoracic lesions, with peripheral lymph node lesions as the most common – in 8 (11.76%) cases. The involvement of the spleen, skin, eyes, and liver was observed only in single cases.

In addition, we studied ABO and Rhesus blood group distribution in patients with pulmonary sarcoidosis. The results obtained are presented in Table IV.

A total of 243 (87.41%) patients were Rhesus positive, while 35 (12.59%) subjects were Rhesus negative.

According to the analysis of patients after the three-month treatment, in Group II (treatment failure), the mean patients' age was 44.3±3.2 years.

In contrast to Group I, where women accounted for 61.71% (29 females), in Group II, there was a male predominance – 13 (61.90%) men (Table V).

According to Table VI, among 58 patients, the treatment was effective in 44 (75.86%) patients with stage II and only 3 (30.00%) patients with stage III pulmonary sarcoidosis.

Seven (70.00%) patients with stage III pulmonary sarcoidosis were GC-resistant.

After the three-month treatment, the frequency of extrapulmonary manifestations was evaluated in patients of both groups (Table VII).

There were only two cases of extrathoracic lesions among patient with favorable treatment outcomes (Group I). In Group II, 11 (84.62%) patients were diagnosed with extrathoracic lesions, including peripheral lymph node lesions in 7 (63.62%) cases.

In 7 (43.75%) out of 16 patients diagnosed with obesity before the start of treatment, clinical radiologic findings demonstrated unfavorable treatment outcomes.

In patients with steroid-resistant sarcoidosis (Group II), there was a high frequency of concomitant cardiovascular pathology – 9 (42.86%) cases, while in Group I, cardiovascular diseases developed in 10 (21.28%) cases only (Table VIII).

There was no significant difference in other comorbidities between the studied groups.

Table IV. ABO and Rhesus blood group distribution in patients with pulmonary sarcoidosis (Ivano-Frankivsk region)

Blood group	Number of patients	
	abs.	%
O	19	27.9±5,0
A	28	41.2±6,0
B	14	20.6±5,0
AB	7	10.3±4,0

Table V. Distribution of patients with pulmonary sarcoidosis after the three-month treatment by gender

Gender	Group I (n=47)	Group II (n=21)
Men	18 (38.3±7,0%)	13 (61.9±11,0%)
Women	29 (61.7±7,0%)	8 (38.1±11,0%)

Table VI. Distribution of patients with pulmonary sarcoidosis depending on the radiologic stage of the disease

Stage	Before treatment	After treatment	
		Group I (n=47)	Group II (n=21)
II	58 (85.3±4,0%)	44 (75.9±6,0%)	14 (24.1±6,0%)
III	10 (14.7±4,5%)	3 (30.0±15,0%)	7 (70.0±15,0%)

Table VII. Frequency of extrapulmonary lesions in patients with pulmonary sarcoidosis after the three-month treatment (Ivano-Frankivsk region)

Localization	Detection rate (number of cases)			
	Group I (n=47)		Group II (n=21)	
	abs.	%	abs.	%
Peripheral lymph nodes	1	2.1±2,0	7	33.3±11,0
Spleen	1	2.1±2,0	1	4.8±5,0
Eyes	0	0±2,0	1	4.8±5,0
Skin	0	0±2,0	1	4.8±5,0
Liver	0	0±2,0	1	4.8±5,0

Table VIII. Frequency of comorbidities in patients with active pulmonary sarcoidosis

Comorbidity	Detection rate (number of cases)				
	Group I (n=47)		Group II (n=21)		
	abs.	%	abs.	%	p>0,05
Cardiovascular pathology	10	21.3±6,0	9	42.9±11,0	p>0,05
Respiratory pathology	3	6.4±4,0	2	9.5±7,0	p>0,05
Ophthalmic pathology	7	14.9±5,0	1	4.8±5,0	p>0,05
Gastrointestinal pathology	8	17.0±5,0	3	14.3±8,0	p>0,05
ENT pathology	3	6.4±4,0	0	0±5,0	p>0,05
Urinary system pathology	1	2.1±2,0	1	4.8±5,0	p>0,05
Anemia pathology	1	2.1±2,0	0	0±5,0	p>0,05
Endocrine pathology	3	6.4±4,0	1	4.8±5,0	p>0,05

According to the analysis of ABO and Rhesus blood group distribution in patients with pulmonary sarcoidosis after the three-month treatment, 11 (52.38%) patients of Group II had type B, Rh-positive blood. In Group I, the distribution corresponded to the initial data.

DISCUSSION

Despite the diverse manifestations of pulmonary sarcoidosis, or vice versa, their absence, there are a number of symptoms that occur mainly all together or each separately. According to scientists, there are the following variants of sarcoidosis clinical course: spon-

taneous regression, regression on the background of treatment, stabilization, progression, fluctuating course, and relapse. We also agree that clinical presentations of sarcoidosis are diverse, ranging from asymptomatic, incidental findings to organ failure [2-3, 5, 11].

According to the authors clinical picture is variable and depends on the organ(s) primarily affected, with non-specific symptoms of respiratory involvement (dry cough, dyspnoea, fatigue and chest pain representing the most common clinical presentation) [3, 5]. In our patients coughing was the most common subjective symptom accompanied by sputum production; dyspnea, shortness of breath.

According to current literature, male gender and age over 40 years are prognostic factors predicting unfavorable outcomes of sarcoidosis. In the cohort study collected between 1996 and 2020 there was a predominance of men. They presented with more severe disease at a younger age, while women more often were found to have involvement of the skin and salivary glands [12]. Among patients under our study, in Group II (treatment failure), the mean patients' age was (44.3 ± 3.2) years, while in Group I (treatment success), it was (37.6 ± 2.8) years. In contrast to Group I, where women accounted for 61.71%, in Group II, a male predominance was observed – 61.9 %.

The highest rate of unfavorable treatment outcomes after the three-month therapy was observed in patients with stage III pulmonary sarcoidosis (70.0%). In 44 (75.9%) patients with stage II and only 3 (30.0%) patients with stage III pulmonary sarcoidosis, the treatment was effective.

Chronic pulmonary sarcoidosis is a systemic disease with typical pulmonary manifestations coexisting with a variety of extrapulmonary lesions affecting its clinical course. In 3 large study cohorts (ACCESS, MUSC, and TTS), the lung was the organ most affected by sarcoidosis (95%, 89%, and 99%, respectively), followed by the skin, eyes, and peripheral lymph nodes [5, 10, 11].

In the works of Magdalena Harasimowicz, Emily GIlbert, Sarah Yi, the lungs, lymph nodes, eyes, skin, liver, joints, and spleen were most often affected when sarcoidosis was detected for the first time. Involvement in the process of the spleen, skin, eyes and liver was observed only in isolated cases [9]. Among patients under our study, there were 13 cases of extrathoracic lesions, with only two patients in Group I (treatment success). In Group II, there were 11 (84.6%) cases of extrathoracic lesions, with peripheral lymph node lesions as the most common - 7 (63.6%) cases.

According to the literature cardiovascular disease is one of the leading causes of morbidity and mortality worldwide in the general population and the second most frequent cause of death in patients with sarcoidosis [5]. Nowadays it is critically important to find optimal strategies in identifying patients at high risk of developing od the disorder. Comorbid conditions often cause difficulties in the differential diagnosis of pulmonary sarcoidosis and can exacerbate the course of the disease. It is worth noting that in patients with treatment failure (Group II), there was a high frequency of concomitant cardiovascular pathology – 9 (42.9%) cases, while in Group I, cardiovascular diseases developed in 10 (21.3%) cases only. For 7 (43.75%) out of 16 patients diagnosed with obesity before the start of treatment, therapy was ineffective.

Analysis of ABO and Rhesus blood group distribution showed that treatment failure was observed in patients with type B, Rh-positive blood.

Summarizing the results of the study, we can conclude that currently sarcoidosis is considered a widespread, complex, systemic disease that affects the entire human body, and at the current stage, the effectiveness of controlling the progression of this disease does not satisfy either patients or doctors, which coincides with the conclusions of colleagues in this field.

CONCLUSIONS

1. Dyspnea (86%), coughing (67%), general weakness and fatigue (29%) on the background of maintaining the indicators of lung tissue density at the level of -893.5 HU and above according to the chest CT imaging represented the three-month treatment failure.
2. Prognostic criteria for the development of GC-resistant pulmonary sarcoidosis could be: stage III disease; age (44.3±3.2) years; gender - male with B positive blood type; extrapulmonary manifestations (lesions of peripheral lymph nodes were seen in 64% of cases); concomitant cardiovascular pathology.

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ORIGINAL ARTICLE

STUDY OF POPULATION ADHERENCE TO COVID-19 VACCINATION

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ABSTRACT

The aim: To study population adherence to COVID-19 vaccination and to identify factors influencing people's decision to vaccinate, and to develop of possible ways to increase the rate of COVID-19 vaccination.

Materials and methods: Individuals of different social groups were surveyed about adherence to COVID-19 vaccination.

Results: Results of the study are the determination of the factors influencing the decision on vaccination. The most important were the opinion that the vaccine is not safe; fear of developing a side effect after vaccination and opinions about the low effectiveness of the vaccine. However, the almost 53% of unvaccinated persons doubted their decision or even expressed a desire to be vaccinated in the future. A possible way to increase the population's adherence to vaccination will be to increase awareness of the effectiveness and safety of vaccination.

Conclusions: The results revealed the factors that influenced the positive and negative decision about COVID-19 vaccination, which made it possible to propose ways to increase the population's adherence to vaccination.

KEY WORDS: vaccination, adherence, COVID-19

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INTRODUCTION

In March 2020, the World Health Organization (WHO) declared a pandemic of a new disease – the coronavirus disease. It was named COVID-19. The disease was first discovered in the Chinese province of Wuhan, but it spread very rapidly in different countries of the world. Almost 778 million people have fallen ill in the world, and more than 6.9 million of them have died as of May 2023 [1]. In Ukraine, since the beginning of the coronavirus pandemic, more than 5.5 million people have fallen ill, more than 115 thousand have died [2]. The COVID-19 pandemic has made humanity remember how dangerous and destructive outbreaks of infectious diseases can be. In such cases, vaccines become the only hope for people to return to normal life. While scientists are actively working on the creation of drugs against COVID-19, the WHO has recognized vaccination as the most effective method of preventing the spread the COVID-19 pandemic. The development of vaccines had a rather difficult and thorny path. But thanks to unprecedented investment and global collaboration, scientists have been able to develop COVID-19 vaccines in record time, following to strict standards based on the principles of evidence-based medicine.

Vaccination started at different times in different countries. Great Britain became the first country to start vaccination in December 2020 [3]. In Poland, vaccination began on December 28, 2020 [4], on February 24, 2021, coronavirus vaccination began in Ukraine [5]. Unfortunately, vaccination rates at the beginning of its implementation were not the same throughout the world, and were unsatisfactory in most countries. As of March 06, 2023, more than 16.2 million people have been fully vaccinated in Ukraine. Meanwhile, 260183 people received a booster dose [6].

Researching the factors influencing people's decision to get coronavirus vaccine will help identify barriers that prevent vaccination. Understanding the factors that promote or hinder vaccination can be a crucial step in making an effective impact on increasing vaccination rates.

THE AIM

Our study was aimed at adherence to COVID-19 vaccination. To reach these objectives the following tasks were set: studying of socio-demographic characteristics of vaccinated and unvaccinated persons; determination of

the factors influencing the decision on vaccination or its refusal; development of recommendations on possible ways to increase the rate of coronavirus vaccination based on the received data.

MATERIALS AND METHODS

The study was conducted in October-November 2021 on the territory of Kharkiv and the Kharkiv region of Ukraine. The survey was conducted by distributing printed questionnaires to visitors to pharmacies, in which the respondents independently gave written answers to the questions. 316 people with different socio-demographic status participated in the survey. The criteria for inclusion in the survey were age over 18 years (as of the beginning of 2021 in Ukraine, COVID-19 vaccination was allowed for adults aged 18 and over; from 16 years for the vaccine from Pfizer/BioNTech + Fosun Pharma [7]). Exclusion criteria from the survey were unwillingness to participate in the survey, pregnant women

To reach the aim of the research, the poll method using anonymous questionnaires was used. Open, closed and dichotomous questions were used in the questionnaire [8]. The researchers developed a questionnaire consisting of 5 sections. The first section contained questions about demographic data of respondents: gender, age, marital status, education, social status. The second section included questions about life history: whether they have had a coronavirus disease or chronic diseases. In the third section, a dichotomous question was asked whether respondents were COVID-19 vaccinated. Then, depending on their answer "yes" or "no", the respondents continued to answer questions about the reasons for consent or refusal of vaccination, determination of conditions for possible consent to vaccination. Statistical characteristics of the variables were presented using percentages, arithmetic means (M) ± standard deviations (SD). To confirm the proposed hypothesis of the influence of various factors on adherence to COVID-19 vaccination, a Pearson (r) correlation was carried out. To estimate the frequency of occurrence of the factor, the "Fisher's angular method" (the φ method) was used. The significance level was set at ($p \leq 0.05$) for all statistical procedures. All methods applied during the study complied with requirements of the Helsinki Declaration of the World Medical Association.

RESULTS

316 people, 181 female and 135 male, took part in the survey on attitudes to COVID-19 vaccination, which was 57.27% and 42.73%, respectively. The average age of respondents was 46.37±14.99 years. All study participants were divided into two groups depending on whether they had been COVID-19 vaccinated. The Group I consisted of persons who received COVID-19 vaccination. This group included 178 people, among whom 68.54% were female and 31.46% were male. The average age of vaccinated persons was 45.58±12.63 years. The Group II included persons who refused COVID-19 vaccination. This group consisted of 138 respondents, among whom 62.32% were female and 37.68% were male. The average age of non-vaccinated persons was 48.41±17.61 years. In both groups, a statistical analysis was conducted according to demographic indicators (age classification [9], marital status, education, social status). According to the WHO age classification, both groups were dominated by young and middle aged individuals (Fig 1). Married people predominated in both groups (Fig 2). By social status, the largest share in both groups was represented by persons who are permanently employed (Fig 3). According to the level of education, persons with higher education predominated in the Group I, persons with secondary special education in the Group II (Fig 4). People with a humanitarian education predominated in the Group I, and with a technical education in the Group II. Based on the results of the correlation analysis, we established the following correlational dependencies: a weak negative significant correlation between the vaccination availability in people and their age ($r = -0.06$; $p<0.05$), between vaccination and people's social status ($r = -0.266$; $p<0.05$); weak positive significant correlation ($r = 0.217$; $p<0.05$) between the level of education of people and the availability of vaccination for them.

The type of vaccine that people received was determined (Fig 5). The obtained data indicate that the majority of people were vaccinated with the COMIRNATY COVID-19 vaccine; 25.84% and 21.34% were vaccinated with the COVISHIELD COVID-19 vaccine and CoronaVac COVID-19 vaccine, respectively, the smallest number of respondents received the Moderna COVID-19 vaccine. Such results are correlated with the number of vaccines of various types that Ukraine received to vaccinate the population against the coronavirus disease. Vaccinated persons answered questions about the choice of vaccine. 79.78% of respondents answered they were vaccinated with the vaccine that was available in the vaccination office, 11.24% followed the doctor's recommendations regarding the choice of the COVID-19 vaccine, and 8.98% listened to the recommendations of friends.

During the study, the influence of the experienced coronavirus disease and chronic diseases among the respondents on the decision on vaccination was researched. In the Group I, individuals who suffered from

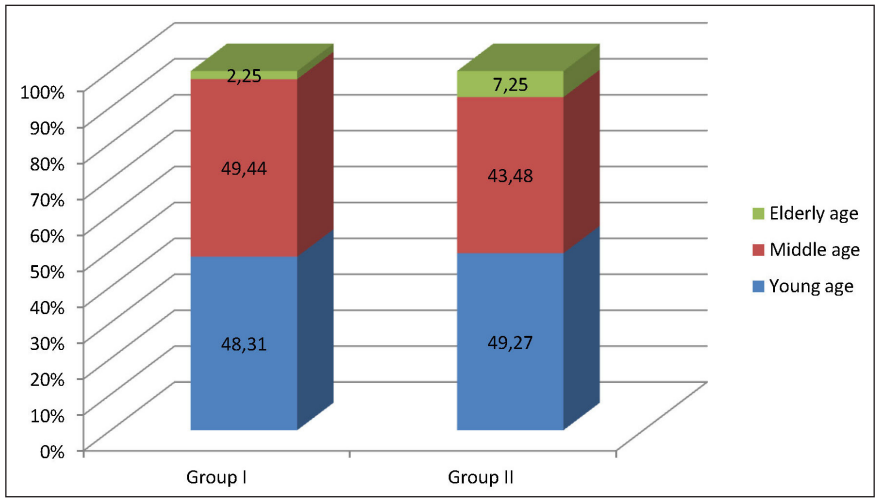

Fig. 1. Age classification of interviewed persons

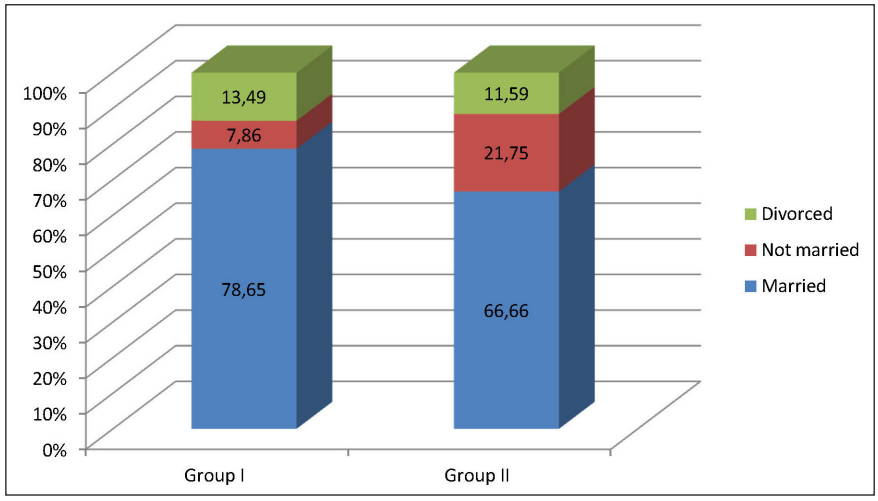

Fig. 2. Marital status of the interviewed persons

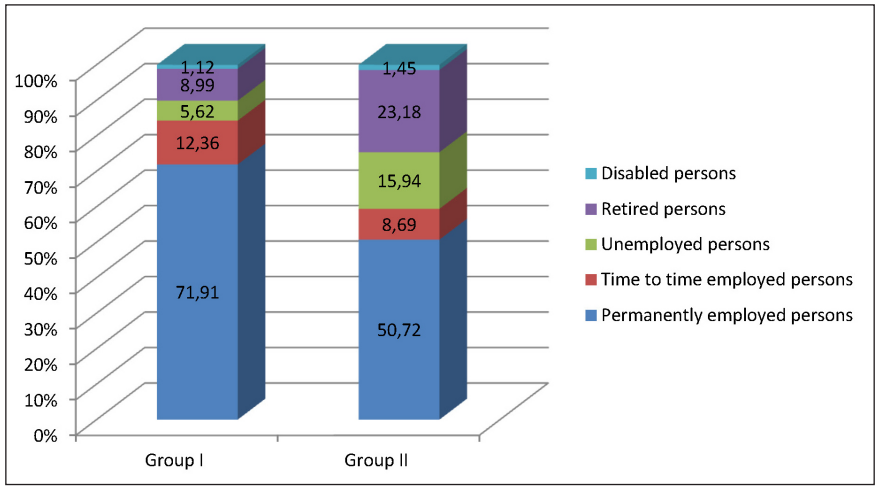

Fig. 3. Social status of the interviewed persons

the coronavirus disease predominated (69.7%). In the Group II, on the contrary, there was a predominance of persons who did not suffer from the coronavirus disease (76.81%). People who suffered from COVID-19 in the Group II made up less than a quarter of the respondents – 23.19%. A negative weak verified correlation was established ($r = -0.104$; $p<0.05$) between the presence of vaccination in people and the presence of chronic diseases in them. Unfortunately, such a correlation indicates a low adherence to vaccination of persons with chronic diseases in contrast to persons without chronic diseases.

It is necessary to understand what exactly influenced the decisions of people who have already been vaccinated in order to develop methods to encourage people to be more actively vaccinated. During the study, the factors that influenced the respondents' positive decision to receive the COVID-19 vaccination were analyzed. The results obtained in the present study indicate that the

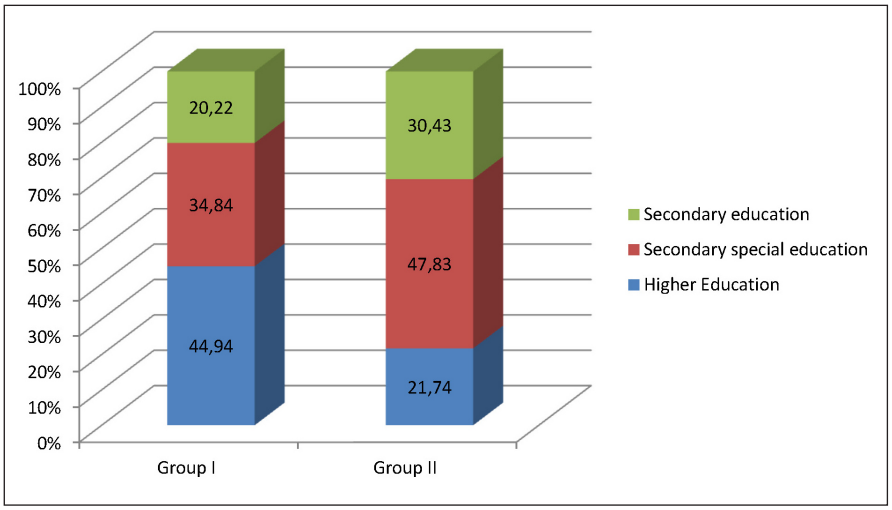

Fig. 4. The level of education of the interviewed persons

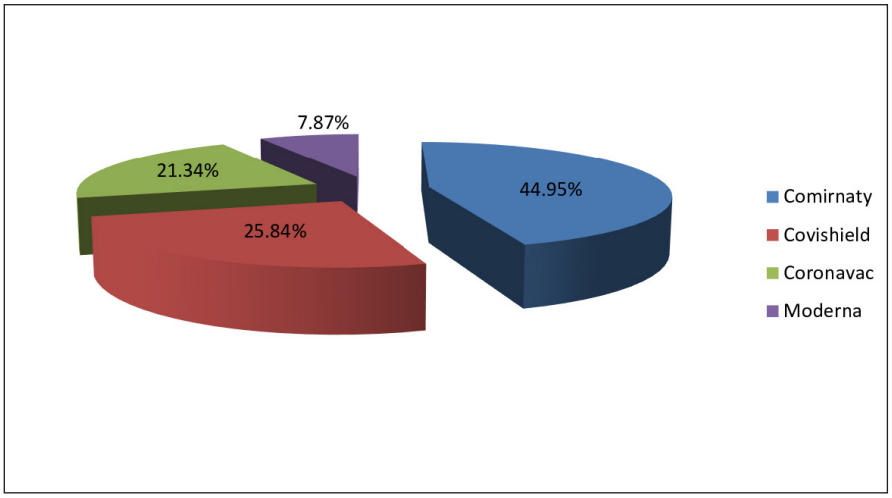

Fig. 5. Distribution of receiving of various types of COVID-19 vaccines

vast majority of people are informed about the necessity and effectiveness of COVID-19 vaccinations. Almost 43% of respondents believe that vaccination will protect them from the disease. Another 28.1% decided to get vaccinated after the illness of close relatives or acquaintances. All of this indicates that people have seen firsthand the hard course and consequences of the coronavirus disease and decided to protect themselves. Almost 13.5% decided on vaccination after the recommendation of a doctor or pharmacist. At the same time, the level of confidence in such recommendations was significantly ($p<0.05$) higher in the Group 1 of vaccinated persons. And this indicates a certain trust in the recommendations of doctors and pharmacists, on the other hand, the percentage of these people is very low. Another nearly 13.5% were vaccinated in order to be able to work permanently, and about 8% were vaccinated in order to be able to travel freely. This suggests that certain administrative levers can increase the percentage of vaccinated individuals, but not significantly.

In order to assess the reliability of the influence of each factor in different population groups, an analysis was carried out using the «Fisher's angular method» (φ method) (Tables I, II, III). The obtained results showed that the opinion that vaccination will protect against the disease was significantly more frequent among the employed than among the unemployed ($\varphi emp. = 2.078$; $p \leq 0.05$) and among retired ($\varphi emp. = 2.17$; $p \leq 0.05$). This indicates a higher responsibility for their health of people who are forced to provide for themselves financially. The frequency of adherence with the doctor's recommendations about vaccination was significantly more frequent in the married than in the single ($\varphi emp. = 2.137$; $p \leq 0.05$) and divorced ($\varphi emp. = 2.399$; $p \leq 0.05$). Married people may visit doctors more often, for example with children, and follow their recommendations better. In addition, married people have a social responsibility for family members and want to protect them. It was clear that the requirement for vaccination at work had a significantly more frequent impact on vaccination among the employed than among the unemployed ($\varphi emp. = 2.433$; $p \leq 0.05$) and among retired ($\varphi emp. = 3.402$; $p \leq 0.05$). The ability to travel freely influenced the decision to get vaccinated significantly more often among young

Table I. The frequency of factors that influenced the positive decision about COVID-19 vaccination in different gender and age groups

№	Factors influenced the decision to get vaccinated	Male/ Female		Young age/ Elderly age		Middle age/ Elderly age		Young age/ Middle age	
		$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p
1	I believe that vaccination will protect me from the disease	1.027	0.152	0.863	0.194	1.171	0.121	0.416	0.339
2	My relatives (employees, familiars) were seriously ill (died) from the coronavirus disease, so I decided to get vaccinated	0.303	0.380	0.566	0.286	0.949	0.171	0.518	0.302
3	I was recommended to get vaccinated by a doctor	0.126	0.45	0.621	0.267	0.073	0.471	0.741	0.229
4	I have to get vaccinated because it is required at the workplace	0.398	0.345	0.073	0.471	1.327	0.092	1.891	0.029
5	I was vaccinated to be able to travel freely	1.304	0.096	2.136	0.016	1.327	0.092	1.093	0.137

Table II. The frequency of factors that influenced a positive decision to get COVID-19 vaccinated depending on marital status

№ п/п	Factors influenced the decision to get vaccinated	Married/ Not married		Married/ Divorce		Not married/ Divorce	
		$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p
1	I believe that vaccination will protect me from the disease	0.439	0.330	0.703	0.241	0.151	0.440
2	My relatives (employees, familiars) were seriously ill (died) from the coronavirus disease, so I decided to get vaccinated	0.445	0.328	0.284	0.388	0.561	0.287
3	I was recommended to get vaccinated by a doctor	2.137	0.016	2.399	0.008	0.0	0.5
4	I have to get vaccinated because it is required at the workplace	0.069	0.472	0.479	0.316	0.287	0.386
5	I was vaccinated to be able to travel freely	0.535	0.296	0.184	0.427	0.561	0.287

Table III. The frequency of factors that influenced a positive decision to get COVID-19 vaccinated depending on employment

№	Factors influenced the decision to get vaccinated	Permanently employed persons/ Time to time employed persons		Permanently employed persons/ Unemployed persons		Permanently employed persons/ Retired persons	
		$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p
1	I believe that vaccination will protect me from the disease	2.078	0.019	1.493	0.0678	2.17	0.015
2	My relatives (employees, familiars) were seriously ill (died) from the coronavirus disease, so I decided to get vaccinated	0.85	0.198	0.565	0.286	0.599	0.2745
3	I was recommended to get vaccinated by a doctor	0.346	0.3645	0.963	0.1678	0.931	0.176
4	I have to get vaccinated because it is required at the workplace	0.0	0.5	2.433	0.0456	3.402	0.0003
5	I was vaccinated to be able to travel freely	1.418	0.078	2.057	0.020	2.281	0.011

people than among the elderly (φemp. = 2.136; p≤0.05) and among working people than among retired (φemp. = 2.281; p≤0.05). This shows that young, working people have more opportunities to travel and do not want to lose this opportunity due to illness. According to other population groups, no significant difference between the frequencies of influence of various factors on the decision about vaccination was found.

Table IV. Factors that influenced respondents' decision not to COVID-19 vaccinate

№	Factors that influenced respondents' decision not to COVID-19 vaccinate	Number of patients
1	I'm afraid of the side effect of the vaccine	36.23%
2	I think that vaccination is not effective	26.08%
3	I think that the vaccine is not safe	42.03%
4	I don't know anything about vaccination	7.25%
5	I can't get to the vaccination point	2.9%
6	I don't know where get vaccinated	1.45%
7	I'm afraid of any injections	4.35%
8	I don't vaccinate because of religious reasons	1.45%
9	I've already had COVID-19 and have antibodies to coronavirus	13.04%
10	I think I have a contraindication to vaccination	11.59%
11	I don't need vaccination because I follow the quarantine rules	11.59%
12	I don't need vaccination because I am young and healthy	13.04%
13	I don't want to get vaccinated	1.45%

Table V. The frequency of factors that influenced respondents' decision not to COVID-19 vaccinate depending on gender, age and marital status

№	Factors that influenced respondents' decision not to COVID-19 vaccinate	Male/ Female		Young age/ Middle age		Middle age / Elderly age		Young age/ Elderly age		Married/ Not married		Married/ Divorce	
		$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p
1	I'm afraid of the side effect of the vaccine	0.629	0.265	0.358	0.360	0.392	0.347	**0.074**	0.470	0.085	0.466	0.273	0.392
2	I think that vaccination is not effective	0.395	0.346	0.947	0.172	0.682	0.248	0.295	0.384	0.915	0.180	0.653	0.257
3	I think that the vaccine is not safe	0.028	0.489	1.547	0.061	1.399	0.081	0.087	0.465	2.375	0.009	2.072	0.019
4	I don't know anything about vaccination	1.322	0.093	1.769	0.038	0.615	0.269	3.163	0.001	1.995	0.023	0.355	0.361
5	I can't get to the vaccination point	0.545	0.293	1.769	0.038	0.545	0.293	1.559	0.059	1.145	0.126	0.85	0.198
7	I'm afraid of any injections	0.282	0.389	1.769	0.038	0.545	0.293	1.559	0.059	2.347	0.009	1.834	0.033
9	I've already had COVID-19 and have antibodies to coronavirus	0.406	0.342	1.444	0.074	1.358	0.087	0.01	0.496	1.049	0.147	1.713	0.043
10	I think I have a contraindication to vaccination	0.604	0.273	1.155	0.124	0.439	0.330	2.118	0.017	2.153	0.016	0.629	0.265
12	I don't need vaccination because I follow the quarantine rules	0.859	0.195	0.422	0.336	1.672	0.047	2.865	0.002	1.746	0.040	0.984	0.162
13	I don't need vaccination because I am young and healthy	0.385	0.350	2.799	0.002	0.0	0.5	3.659	0.	0.038	0.485	0.121	0.452
14	I don't want to get vaccinated	1.665	0.048	0.968	0.166	0.0	0.5	1.265	0.102	1.145	0.126	0.85	0.198

The next stage of the research was to study the factors influenced the decision of the interviewed persons not get COVID-19 vaccine (Table IV).

The obtained data made it possible to establish the factors that most prevented vaccination: the opinion that the vaccine is not safe (42.03%); fear of developing a side effect after vaccination (36.23%) and opinions about the low effectiveness of the vaccine (26.08%). About 13% of respondents believe that they will be protected by the antibodies they have after suffering from the coronavirus disease and by their young age. 11% of respondents refused vaccination because they have contraindications to vaccination, and also consider that compliance with quarantine regulations

Table VI. The frequency of factors that influenced respondents' decision not to COVID-19 vaccinate depending on education and employment

№	Factors that influenced respondents' decision not to COVID-19 vaccinate	Higher Education/ Secondary special education		Higher Education/ Secondary education		Permanently employed persons/ Time to time employed persons		Permanently employed persons/ Unemployed persons		Permanently employed persons/ Retired persons	
		$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p	$\varphi_{емп.}$	p
1	I'm afraid of the side effect of the vaccine	0.305	0.380	0.642	0.260	0.628	0.265	0.387	0.350	0.11	0.456
2	I think that vaccination is not effective	1.499	0.067	0.121	0.452	0.998	0.159	0.712	0.239	0.386	0.350
3	I think that the vaccine is not safe	0.853	0.197	0.642	0.260	0.177	0.430	0.881	0.190	0.177	0.430
4	I don't know anything about vaccination	1.982	0.024	1.308	0.095	1.69	0.045	2.24	0.013	0.499	0.309
5	I can't get to the vaccination point	1.138	0.128	1.308	0.095	0.0	0.5	0.0	0.5	2.267	0.012
7	I'm afraid of any injections	1.22	0.111	2.173	0.015	0.746	0.228	0.767	0.221	1.221	0.111
9	I've already had COVID-19 and have antibodies to coronavirus	0.917	0.179	0.332	0.369	1.69	0.0455	2.24	0.013	0.026	0.490
10	I think I have a contraindication to vaccination	0.043	0.483	1.454	0.073	1.301	0.097	1.38	0.084	0.663	0.254
12	I don't need vaccination because I follow the quarantine rules	0.043	0.483	0.76	0.223	1.059	0.145	1.701	0.044	1.733	0.042
13	I don't need vaccination because I am young and healthy	0.062	0.475	0.314	0.377	0.203	0.420	0.269	0.394	2.765	0.003
14	I don't want to get vaccinated	1.138	0.128	0.0	0.5	0.746	0.228	0.989	0.161	1.221	0.111

is sufficient to protect against the disease. About 7% of respondents do not know anything about the COVID-19 vaccination (Table V).

The application of "Fisher's angular method" (φ method) made it possible to evaluate the reliability of the influence of each factor on the negative decision about vaccination in different population groups. It was established that the opinion that the vaccine is not safe was significantly higher among married people than among single people (φemp. = 2.375; p≤0.05) and then among divorced people (φemp. = 2.072; p≤0.05). This indicator shows a higher responsibility for their own health, since married people are responsible not only for themselves, but also for family members. The frequency of lack of knowledge about vaccination was significantly higher in people of middle (φemp. = 1.769; p≤0.05) and elderly (φemp. = 3.163; p≤0.05) age than in young people; significantly more in married than in single (φemp. = 1.995; p≤0.05); significantly more in people with secondary special education than with higher education (φemp. = 1.982; p≤0.05); it is significantly more among those who work from time to time (φemp. = 1.69; p≤0.05) and the unemployed (φemp. = 2.24= ; p≤0.05) than among those who work. The obtained result demonstrates that a number of different factors influence the level of people's knowledge about vaccination. It was clear that the influence of the factor of impossibility to reach the vaccination point was significantly greater in middle-aged people than in young people (φemp. = 1.769; p≤0.05) and in retired than in working people (φemp. = 2.267; p≤0, 05). Fear of any injections occurred to significantly more often in middle-aged people than in young people (φemp. = 1.769; p≤0.05); more in single (φemp. = 2.347; p≤0.05) and divorced (φemp. = 1.834; p≤0.05) than in married; more in people with higher education (φemp. = 2.173; p≤0.05) than with secondary education. The factor of previous illness occurred significantly more in married (φemp. = 1.713; p≤0.05) and unmarried (φemp. = 2.282; p≤0.05) than in divorced people; significantly more among working people than among occasional workers (φemp. = 1.69; p≤0.05) and retired (φemp. = 2.24; p≤0.05). Such a show suggests that people, who have more contacts have a higher risk of COVID-19, but believe that they will not get sick again and don't need to be vaccinated. Married and elderly people are significantly more thinking that they have contraindications to vaccination than young people (φemp. = 2.118; p≤0.05) and single (φemp. = 2.153;

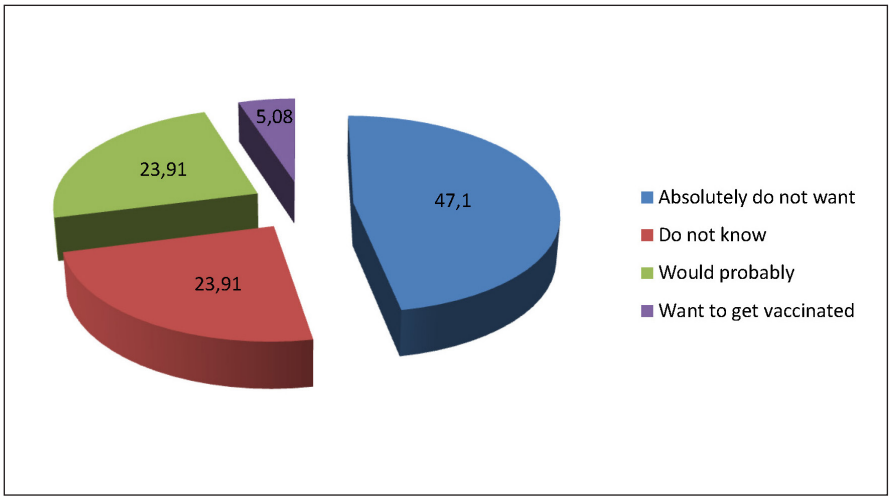

Fig. 6. Distribution of respondents depending on their willingness to get COVID-19 vaccinated

p≤0.05). Older people have more chronic diseases and therefore more often think about contraindications to various interventions, including vaccinations. Young and middle-aged people consider adherence with quarantine rules to be sufficient protection against COVID-19 and therefore refuse vaccination respectively (φemp. = 2.865; p≤0.05) and (φemp. = 1.672; p≤0.05) significantly more often than elderly. The same opinion is significantly held by single more often than married (φemp. = 1.746; p≤0.05); unemployed (φemp. = 1.701; p≤0.05) and retired (φemp. = 1.733; p≤0.05) than employed people. Such results revealed that the factor of strict adherence to quarantine rules is influential among different socio-demographic groups. It was clear to find a significantly greater influence of the factor "I don't need vaccination because I am young and healthy" in young people than in middle-aged (φemp. = 2.799; p≤0.05) and elderly people (φemp. = 3.659; p≤ 0.05); in workers than in retired (φemp. = 2.765; p≤0.05). Men expressed unwillingness to get vaccinated significantly more often (φemp. = 1.665; p≤0.05). According to other population groups, no significant difference between the frequencies of influence of various factors on the negative decision about vaccination was found (Table VI).

During the survey, the question of whether non-vaccinated persons still want to get coronavirus vaccinated was clarified (Fig 6).

47.1% of unvaccinated persons categorically refuse vaccination, while 47.82% expressed certain doubts about their decision. And only 5.07% expressed a desire to be vaccinated. An analysis of the conditions under which respondents would make a decision to vaccinate showed that 23.19% of respondents needed time to make a decision after evaluating the consequences and effectiveness of vaccination. 10.15% and 7.25%, respectively, need additional information about COVID-19 vaccines from a doctor and his recommendation to get vaccinated. Also, 7.25% of respondents need a requirement to get vaccinated at the workplace. About 3% simply indicated a lack of desire to get vaccinated.

DISCUSSION

Summarizing the obtained data, it is possible to make a "portrait" of a person who consciously made a choice in favour of or against COVID-19 vaccination. Vaccinated persons are young or middle-aged married women, or retired, or disabled women with higher technical, medical and pharmaceutical education. Non-vaccinated persons are constantly working married women of young age with secondary special humanitarian education. The vast majority of people were vaccinated with the vaccine that was available at the vaccination point, and only about 11% of people were guided by the physician recommendations.

In the study, the most influential factors for a positive decision about vaccination were the protection of vaccination against the disease, the negative experience of ill or deceased relatives (employees, familiars) and the ability to work normally. Similar data were obtained in studies in Ukraine by the Sociological Group "Rating" [10] and the Red Cross Society [11], but the percentage of people who were vaccinated for their own protection was much higher in investigation of the Red Cross Society, the second position was occupied by dependence on the ability to work from vaccination. In studies in other countries health communication regarding safety, side effect, and effectiveness of vaccines also were identified as the most important predictor to convert vaccines to vaccinations **[**12**].** Another study showed that the main factors influencing vaccination decisions were vaccine convenience (95.7%) and physician recommendation (97.3%) [13]. In another study, participants' vaccination rates were associated with the ability to work, source of income, health status, level of

knowledge about COVID-19, and willingness to protect family members **[**14**].**

The influence of gender on the decision to vaccinate turned out to be ambiguous. In our study, men expressed unwillingness to get vaccinated significantly more often. In the study by Fischbach L, Civen R, Boyd H et al. "gender" had a non-significant correlation with the adherence with vaccination [11]. In another study, acceptance of vaccination was lower in women than in men [15].

Various studies have shown that young age, higher education, students, unmarried marital status most often influence the vaccination rate [13]. In addition, it was shown that uptake of COVID-19 vaccination in older patients were also mediated by practical issues such as access and affordability [16].

The most common reasons for refusing vaccination were fears of a side effect of the vaccine, opinions about the ineffectiveness and danger of vaccines against COVID-19, as well as a previous illness. In the study of the Red Cross Society of Ukraine on the population's perception of vaccination, the leading factor of refusal was the presence of chronic diseases in a person, followed by fear of the ineffectiveness and danger of vaccines [11]. According to the results of another study, the leading claim was that people with chronic diseases should not be vaccinated. A third of respondents believe that those who have got sick the coronavirus do not need to be vaccinated [10]. A study in Australia found that barriers to vaccination included safety and effectiveness concerns, perceived scientific uncertainty, low disease risk perception, and low trust in authorities and other stakeholders [17].

The results of the study showed that among unvaccinated persons, almost 53% doubted their decision or even expressed a desire to get vaccinated in the future, provided that the consequences and effectiveness of the vaccination were evaluated, receiving additional information about the COVID-19 vaccines from the physician and his recommendation to be vaccinated, as well as the requirement get vaccinated at work. Similar results were obtained in another study [10], but it was shown that people are more willing to get vaccinated for free. In the study of the Red Cross Society [11], the main reasons for consent to vaccination were found to be self-protection, a requirement for work, to protect others. The analysis of the factors influenced a positive decision about vaccination showed that most people are informed about the need and effectiveness of COVID-19 vaccinations (only 7% of respondents do not know anything about COVID-19 vaccination, and 3% simply do not want to be vaccinated). The main reasons for vaccination were the belief in the efficacy of vaccination and the desire to protect oneself from the disease. The recommendations of doctors and pharmacists had a certain influence on a positive decision to get vaccine, at the same time; administrative measures had an influence on a small number of respondents.

The analysis of the conditions under which the respondents would make a decision to vaccinate showed that people need time to make a decision after evaluating the consequences and effectiveness of vaccination, they also need additional information about COVID-19 vaccines from a doctor and his recommendation to vaccinate, or a requirement to vaccinate from an employer.

CONCLUSIONS

According to the results of the work carried out by the researchers, the goals of the study were fully achieved.

1. Socio-demographic characteristics and education levels of vaccinated and non-vaccinated persons were established. There were young and middle-aged married persons who are permanently employed persons and have a higher humanitarian education.
2. Among individuals who have already experienced a coronavirus disease, the adherence of vaccination was significantly ($p<0,05$) higher, while individuals with the presence of chronic diseases had a lower significantly ($p<0,05$) adherence of vaccination. Almost 80% of the surveyed people were vaccinated with the vaccine that was available in the vaccination office.
3. According to the results of the study, it was established that the global perception of COVID-19 vaccinations depends on various factors, each of which reliably prevails in different socio-demographic groups, and is also related to the vaccine itself (its safety, effectiveness, availability). Among the factors that had the significantly ($p<0,05$) greatest positive influence on the adherence with vaccination in different population groups were protection from the disease (in working people), a physician's recommendation to vaccinate (in married people), the requirement to vaccinate at work (in working people) and the opportunity to travel (in young and working persons). There are significant ($p<0,05$) factors of negative adherence with vaccination opinions about the "danger" of the vaccine (in married people), lack of information about vaccination (in middle-aged working married people with secondary special education); impossibility to get to the vaccination point (for middle-aged people and pensioners); fear of any injections (in a single or divorced middle-aged people with higher education); the presence of anti-

bodies after having already experienced COVID-19 (in a married or a single permanently employed persons); the presence of contraindications (for a single elderly people), refusal of vaccination due to follow the quarantine rules (for a single young and middle-aged people, unemployed people and retired); factor "I do not need vaccination because I am young and healthy" (in young working people), lack of desire to be vaccinated (in male).

4. It was determined that more than 50% of unvaccinated people are theoretically ready to be vaccinated, but they need certain conditions: time to make a decision after evaluating the consequences and effectiveness of vaccination, additional information about vaccines and recommendations to be vaccinated from a doctor (sometimes from familiars or relatives) and a requirement to be vaccinated from employers. Vaccinated individuals, compared to non-vaccinated individuals, had significantly ($p<0,05$) greater trust in both physicians and pharmacists as sources of information regarding COVID-19 vaccination.
5. On the basis of the identified factors that influence the respondents' positive decision to vaccinate, and the level of trust in various medical sources of information, in our opinion, the key ways to improve vaccination rates can be done by intensifying educational work by medical and pharmaceutical workers among people (especially those with chronic diseases) in order to increase their awareness of the effectiveness and safety of vaccination.

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ORIGINAL ARTICLE

PECULIARITIES OF PERCEPTION OF NON-VERBAL STIMULI BY PATIENTS WITH SCHIZOPHRENIA: SUBJECTIVE UNDERSTANDING, INTEREST AND EMOTIONS

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ABSTRACT

The aim: The main purpose of this article was to investigate the subjective understanding, interest, and emotional perception of non-verbal stimuli by schizophrenic patients.

Materials and methods: For this study, the clinical interview method was used, in which all questions were presented in Ukrainian. The questionnaire form was divided into three main blocks: socio-demographic, mental anamnesis, and non-verbal stimulus assessment. 50 respondents took part in the study. The experimental group, i.e., respondents with schizophrenia spectrum disorders, made up 58% (n = 29) of the total number of respondents, and the control group, respondents with other diagnoses, made up 42% (n = 21).

Results: The results showed that in both groups the level of abstractness or objectivity of the drawings affected the understanding of what was depicted. Patients with schizophrenia had a better understanding of images that didn't have a single semantic and compositional whole. The abstractness of the drawings and their detailing affected the appearance of interest in the image in people who have disorders on the schizophrenia spectrum. In addition, the more realistic the objects in the picture were, the less interesting it was for people with schizophrenia spectrum disorders.

Conclusions: In conclusion, schizophrenic patients found stereotypical signs of emotions much more easily than respondents with other diagnoses, and facial expressions were the most important factor in determining the emotional component of drawings.

KEY WORDS: visual perception, cognitive impairment, emotional response, mental disorders

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INTRODUCTION

The problem of the subjective perception of the world appears before the sciences, that study human consciousness and psyche – a problematic one, that does not have a single, universal solution. This applies to both healthy people and people who have mental pathologies. Schizophrenia is a mental disorder that affects a person's perception, thinking, and behavior. One aspect of perception that is often impaired in people with schizophrenia is the perception of art. Pictures, in particular, can be difficult to interpret for patients with schizophrenia because of their complexity and the range of emotions they can evoke.

Many scientists have studied the peculiarities of the perception of the surrounding world among people with schizophrenia from different angles: the issue of visual perception [1-3] studies of sound perception [4, 5] the perception of movements [6, 7] the study of the perception of emotions [8, 9] questions about the peculiarities of verbal and non-verbal communication [10-12]. The question of visual perception among patients with schizophrenia is one of the most studied because it is approached from different perspectives, for example, neurophysiological [13, 14], psychological and psychiatric [15, 16], and social rehabilitation [17, 18]. The large number of publications on the mentioned topic is certainly due to its practical significance, because all these approaches are combined to describe the studied phenomenon from different angles.

Visual image perception is an important part of most people's understanding and knowledge of their surroundings [19, 20]. One of the forms of reflecting the subjective perception of the world is creativity. Creativity is an integral and exclusive aspect of the human experience. However, the creation of creative products and their evaluation are based on biological and behavioral processes, that differ between healthy people and those with mental disorders. Therefore,

Table I. Review of socio-demographic data

Groups		Number	Percentage
Gender	Female	9	18%
	Male	41	82%
Age Groups	18-20	6	12%
	21-25	6	12%
	26-45	26	52%
	46-59	9	18%
	60+	3	6%
Education	Full Secondary	6	12%
	Technical	8	16%
	Unfinished higher education	10	20%
	Higher education	26	52%
Marital status	Married	7	14%
	Divorced	6	12%
	Single	35	70%
	Engaged	1	2%
	Widowed	1	2%

creativity can serve as a source of information about how the brain perceives and evaluates the visual world [21, 22]. It is known that schizophrenic people create creative images with visual attributes in composition, i.e., color, shape, and configuration, which are of a very extraordinary nature [23]. It is clear that the evaluation of the products of creativity by patients with schizophrenia can be significantly different from the perception of creativity by healthy people. Given that when diagnosing mental disorders, psychologists and psychiatrists quite often use methods that have non-verbal stimulus material, it is relevant to study the peculiarities of the perception of visual images by mentally ill people.

THE AIM

The main hypothesis of the research is that the respondents who had the same diagnosis as the author of the non-verbal stimuli would on average better subjectively understand what was depicted in the picture compared to the respondents with other diagnoses.

We assumed that each of the several factors would determine the peculiarities of perception of non-verbal stimuli by patients with schizophrenia, therefore outlining the following research objectives: (i) to assess the level of subjective understanding of the pictures among the participants of two groups; (ii) to ascertain the degree of interest demonstrated by respondents in both groups towards the images presented; (iii) to determine the degree of expression of a specific emotion in relation to each image among the participants of two groups.

MATERIALS AND METHODS

The data were collected in a single phase. For this study, the clinical interview method was used, in which all questions were presented in Ukrainian. The research methodology consisted of the following parts: socio-demographic and anamnestic blocks, which included questions about the age of respondents, level of education, marital status, presence or absence of auto-aggressive behavior, presence or absence of disability, diagnosis according to ICD-10, duration of illness, duration of treatment, number of remissions, number of admissions to the hospital per year, taking medications at home, and use of psychoactive substances during the last year. The stimulus material perception block included questions that had an open form or were presented as a semantic differential. The subjects were asked to rate the drawings (Appendix A) on a scale from 1 to 7 according to the following characteristics: understanding of what is depicted in the drawing, interest in the drawing, and correspondence to the emotion that the author of the drawing noted (Fig. 1). In open-ended questions, respondents were asked to describe what they see in the pictures using adjectives and name the picture in one word. The sample was formed by recruiting all patients of the Communal Non-Profit Enterprise Clinical Hospital Psychiatry of the Executive Authority of the Kyiv City Council (Kyiv City State Administration). The

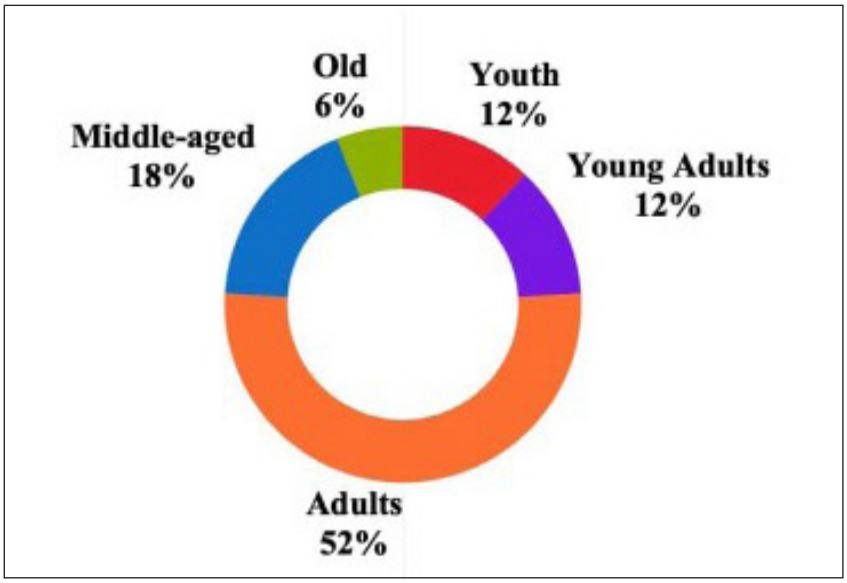

Fig. 1. Distribution of the sample by age

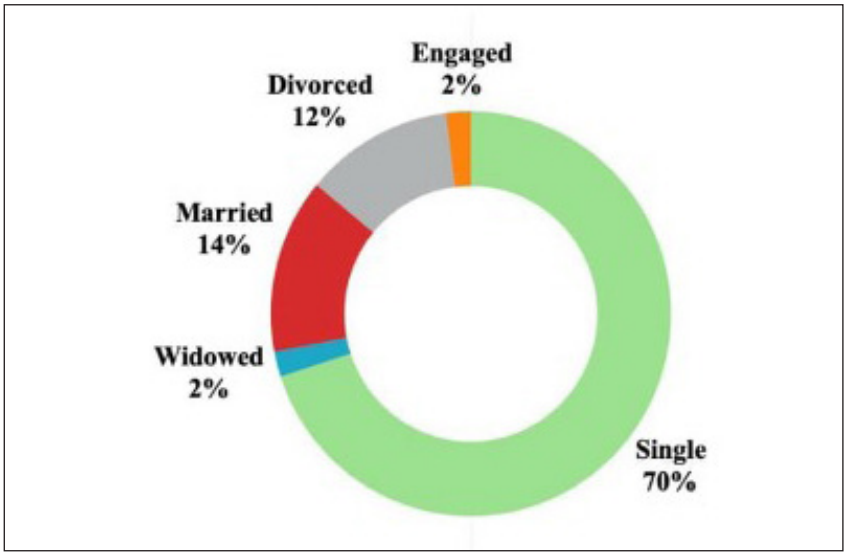

Fig. 2. Distribution of the sample by marital status

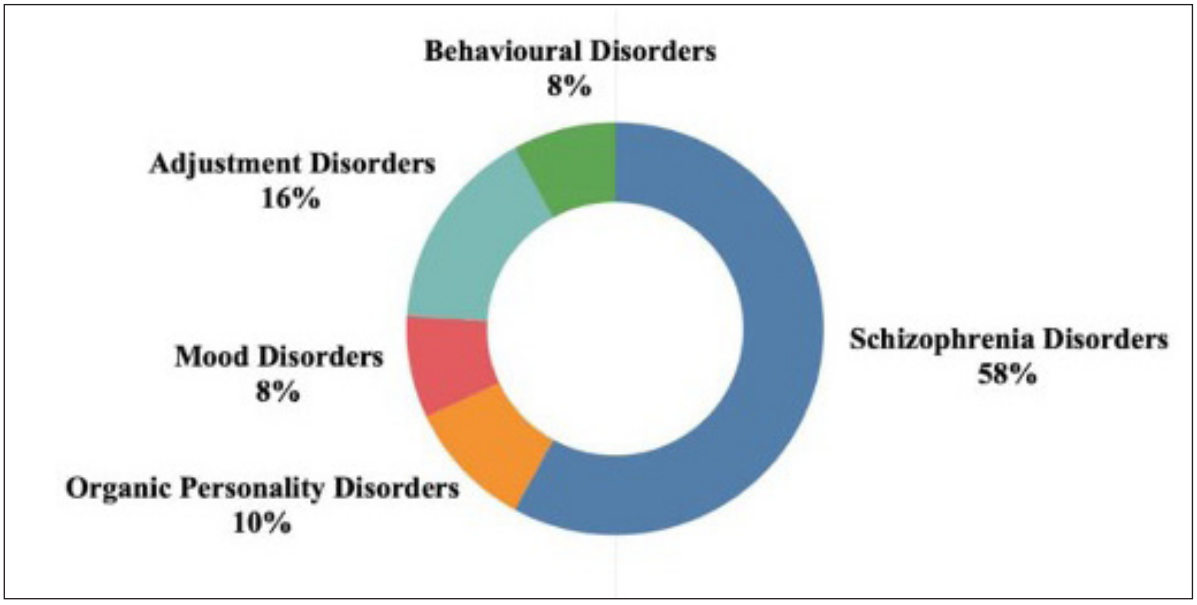

Fig. 3. Distribution of the sample by diagnoses

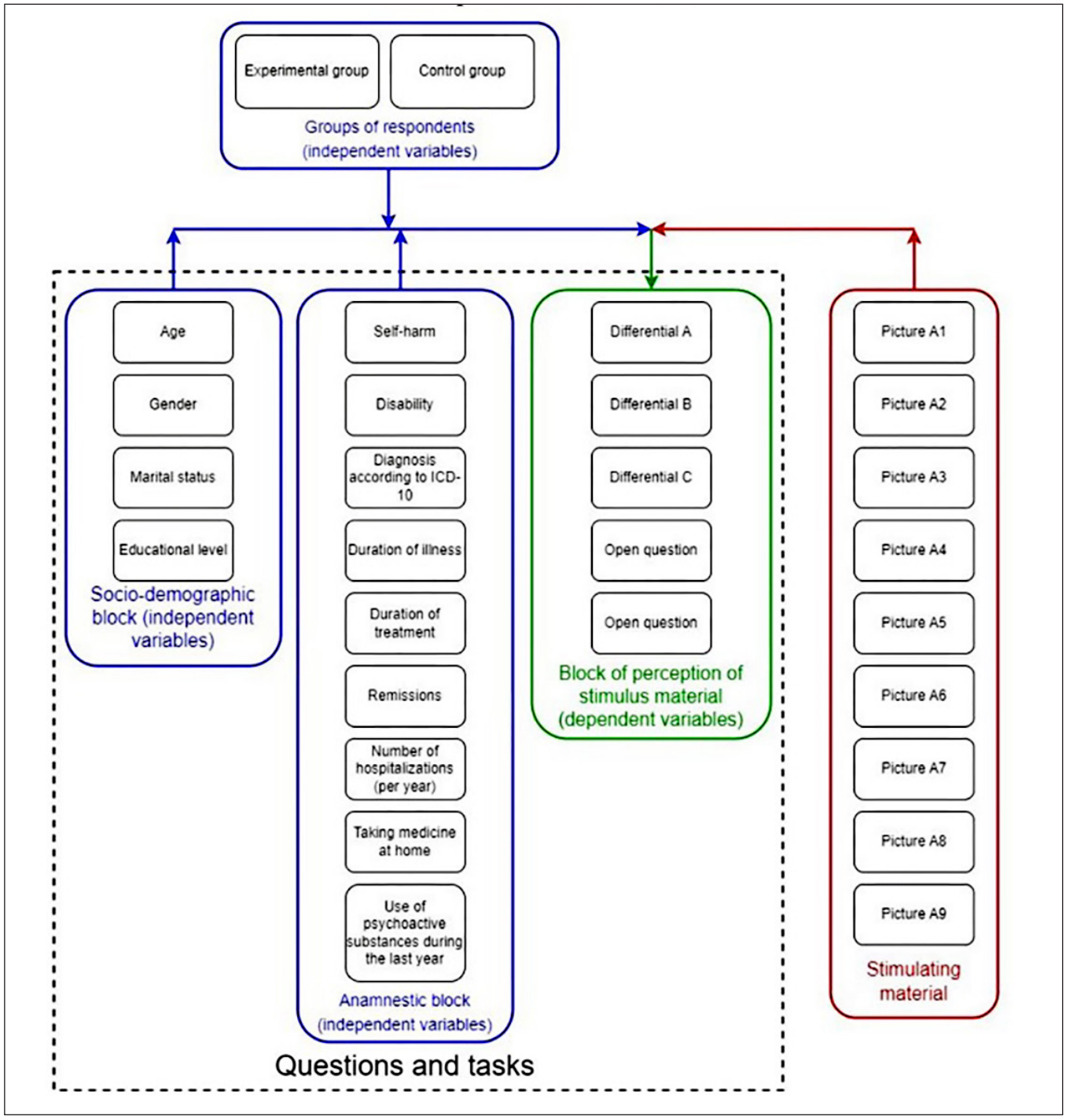

Fig. 4. Conceptual research model

criteria for including respondents in the sample were: the presence of an established psychiatric diagnosis and legal age. The respondents were divided into two groups: in the experimental group, the respondents who had the same diagnosis as the author of the pictures were included (schizophrenia spectrum, F20-F29), while the respondents with other psychiatric diagnoses formed the control group.

It should be noted that during the research, the core quantities which we evaluated (subjective self-assessed understanding, emotion and interest with regard to the presented images) are purely subjective by their nature, and therefore can be measured directly. This is an important property of such quantities which, compared to absolute majority of objective quantities, can be evaluated by using fundamental statistical analysis without necessarily involving established and approved methodologies or questionnaires.

50 respondents took part in the study; 82% of respondents (n = 41) were male, and 18% of respondents

Table II. Frequency distribution of responses in the control and experimental groups according to differential A

Response for differential A	Group number	Counts								
		Pic. A1	Pic. A2	Pic. A3	Pic. A4	Pic. A5	Pic. A6	Pic. A7	Pic. A8	Pic. A9
1	*1*	2	7	0	1	1	1	1	0	0
	2	1	7	2	0	1	0	1	1	0
2	*1*	1	3	2	0	0	0	1	2	2
	2	2	6	0	0	0	2	0	1	2
3	*1*	1	6	5	0	2	0	3	3	3
	2	2	0	0	1	1	1	0	2	1
4	*1*	1	2	6	0	2	4	3	5	1
	2	2	2	5	0	2	0	4	3	0
5	*1*	7	1	0	2	4	3	3	4	4
	2	1	1	1	5	3	0	4	2	5
6	*1*	3	1	4	5	8	4	3	5	4
	2	2	1	3	4	3	3	3	2	3
7	*1*	14	9	12	21	12	17	15	10	15
	2	11	4	10	14	10	14	8	9	9

(n = 9) were female. The average age of the sample was 36.2 years. 70% of respondents (n = 35) indicated that they were single, 14% of respondents (n = 7) indicated that they were married, 12% of respondents (n = 6) were divorced, 2% of respondents (n = 1) were engaged, and 2% of respondents (n = 1) indicated that they were widowed. For the study, the respondents were divided into the five age categories (Figs. 2, 3; Table I).

Overall, 30% of respondents (n=15) noted the presence of auto-aggressive behavior, 70% of respondents (n=35) indicated its absence. 24% of respondents (n=12) had a disability, 76% of respondents (n=38) didn't. The respondents' diagnoses were classified into the following groups: schizophrenia disorders, adjustment disorders, organic personality disorders, mood disorders, and behavioral disorders (Fig. 4).

The duration of the respondents' illness was divided into three time periods: <1 year of illness in 32% of respondents (n = 16), 1–5 years of illness in 22% of respondents (n = 11), and >5 years of illness in 46% of respondents (n = 23). The duration of treatment among respondents was divided into three-time intervals: <1 year of treatment for 40% of respondents (n = 20), 1–5 years of treatment for 26% of respondents (n = 13), and >5 years of treatment for 34% of respondents (n = 17). According to the number of remissions, respondents were divided as follows: 40% of respondents (n = 20) had no remission, 24% of respondents (n = 12) had one remission, 10% of respondents (n = 5) had 2 remissions, 10% of respondents (n = 5) had 3 remissions, 10% of respondents (n = 5) had more than 5 remissions, and 6% of respondents (n = 3) had 4 remissions. 74% of respondents (n = 37) had one admission to the hospital per year, 14% of respondents (n = 7) had two admissions to the hospital per year, 10% of respondents (n = 5) had three admissions to the hospital per year and 2% of respondents (n = 1) had four and more admissions to the hospital per year. 60% of respondents (n = 30) mentioned taking medication at home, and 40% of respondents (n = 20) weren't accepted. 44% of respondents (n = 22) indicated that they used psychoactive substances in the last year, and 56% of respondents (n = 28) didn't.

Statistical analysis was performed using Jamovi [24] and for the data visualization was used the Anaconda v.2022.10, which is a distribution of the Python programming language [25] and was presented in accordance with the research objectives. To (i) assess the level of subjective understanding of the pictures among the participants of two groups; (ii) ascertain the degree of interest demonstrated by respondents in both groups towards the images presented; (iii) determine the degree of expression of a specific emotion in relation to each image among the participants of two groups. Frequency analysis and Spearman's correlation coefficient were used to test the research hypothesis about the presence of systematic differences between the ratings of respondents in the two groups.

The study meets ethical requirements and has received approval from the Ethics and Academic Integrity Commission Kyiv Institute of Modern Psychology and Psychotherapy. Informed consent was obtained from each participant.

Table III. Frequency distribution of responses in the control and experimental groups according to differential B

Response for differential B	Group number	Counts								
		Pic. A1	Pic. A2	Pic. A3	Pic. A4	Pic. A5	Pic. A6	Pic. A7	Pic. A8	Pic. A9
1	*1*	9	4	7	3	4	5	7	3	9
	2	4	4	2	1	1	1	2	3	6
2	*1*	4	4	5	2	2	4	1	4	3
	2	1	2	1	1	1	2	1	3	1
3	*1*	3	3	0	3	3	4	0	3	3
	2	1	2	6	1	2	4	2	4	0
4	*1*	3	2	5	3	1	3	2	4	4
	2	5	5	3	5	2	4	4	2	3
5	*1*	3	4	3	3	4	0	4	7	2
	2	2	3	0	1	3	1	2	1	2
6	*1*	2	0	4	5	4	4	4	3	2
	2	1	2	2	4	4	4	3	2	3
7	*1*	5	12	5	10	11	9	11	5	6
	2	7	3	7	8	7	4	6	5	5

Table IV. Frequency distribution of responses in the control and experimental groups according to differential C

Response for differential C	Group number	Counts								
		Pic. A1	Pic. A2	Pic. A3	Pic. A4	Pic. A5	Pic. A6	Pic. A7	Pic. A8	Pic. A9
1	*1*	4	5	16	4	16	11	3	3	3
	2	5	7	13	1	11	9	0	1	1
2	*1*	1	5	5	1	3	4	0	1	2
	2	2	4	0	2	3	0	1	1	0
3	*1*	6	4	1	0	3	4	2	1	0
	2	2	0	3	1	1	3	1	2	2
4	*1*	4	5	1	3	1	4	0	4	0
	2	2	2	1	4	0	4	2	5	1
5	*1*	4	2	2	4	2	2	1	5	2
	2	0	4	1	4	1	1	3	4	1
6	*1*	1	2	2	6	2	2	8	5	2
	2	4	0	1	3	2	1	3	0	2
7	*1*	9	6	2	11	2	2	15	10	20
	2	6	4	2	6	2	2	10	7	13

RESULTS

We assumed that the respondents in the experimental group, that is, those who had the same diagnosis as the author of the pictures, would subjectively better understand what was depicted in the picture than the respondents who had other diagnoses. Frequency analysis was used to test this hypothesis (Table II). It should be noted that the respondents in both groups considered the most understandable (and least understandable) the same pictures. Respondents in both groups understood Fig. 8 A4 and Fig. 8 A6 the best and Fig. 8 Picture A2 the worst, but in general, respondents with schizophrenia rated their understanding of the pictures better. This shows that if the complexity of

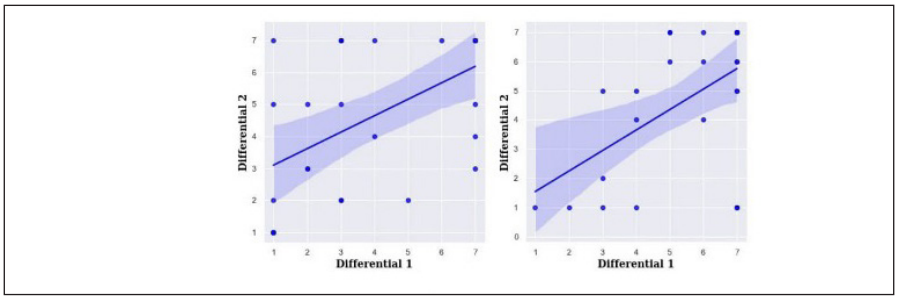

Fig. 5. Visualization of correlations for the experimental group (Picture A2 and Picture A7)

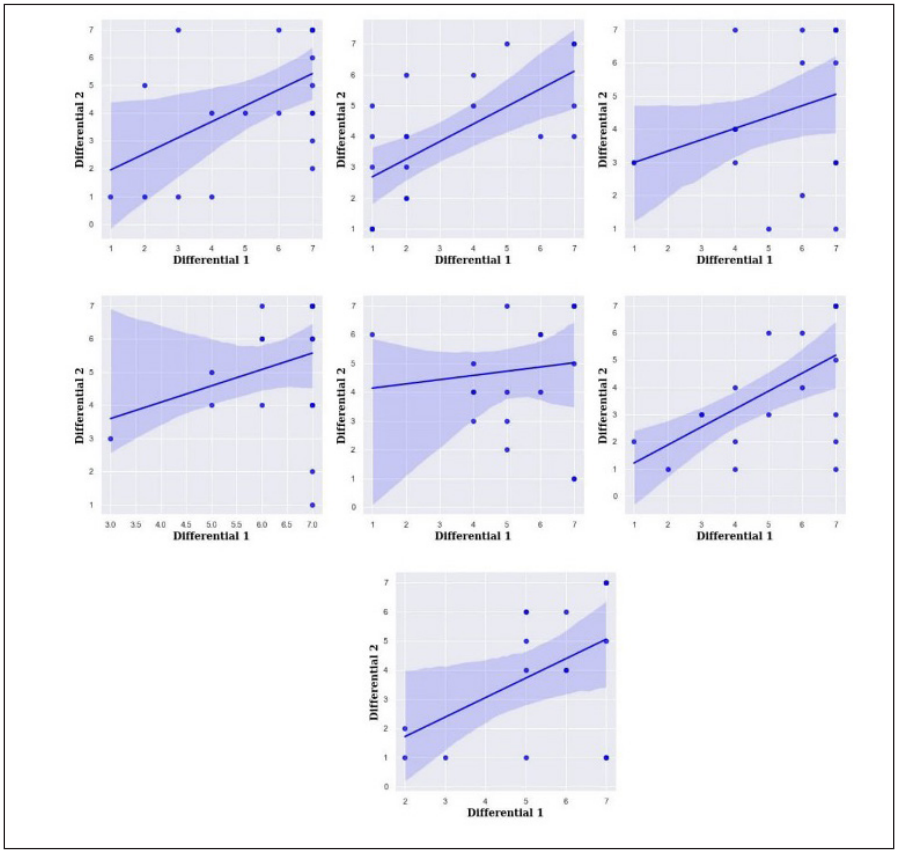

Fig. 6. Visualization of the correlations for the control group (Picture A1, Picture A2, Picture A3, Picture A4, Picture A7, Picture A8 and Picture A9)

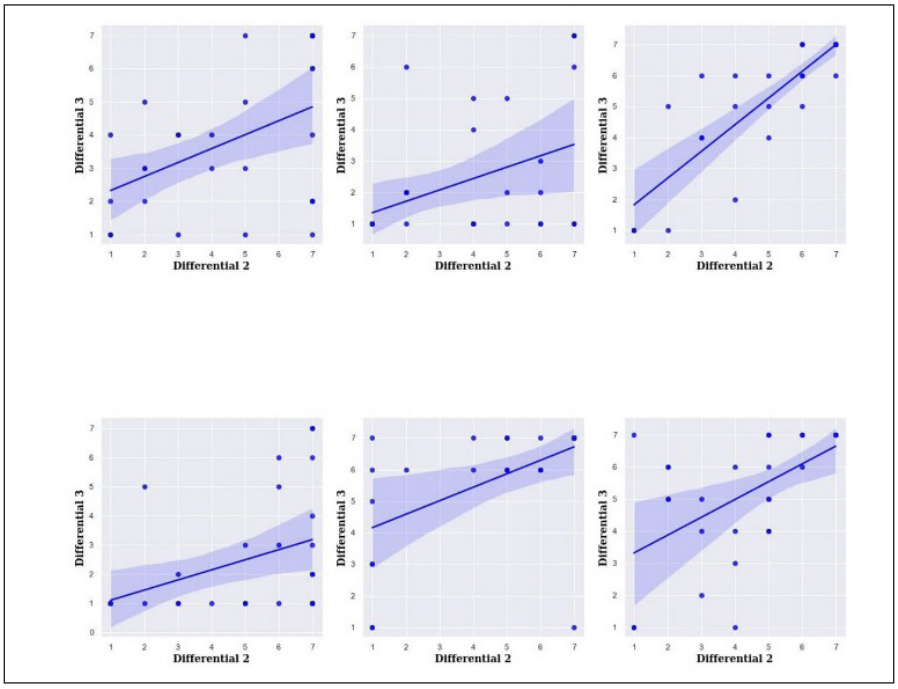

Fig. 7. Visualization of correlations for the experimental group (Picture A2, Picture A3, Picture A5, Picture A7 and Picture A8)

Table V. Correlation analysis (Spearman's method) for differential A and differential B within experimental and control groups

Picture Number	Group 1		Group 2	
	Spearman's correlation	p-value	Spearman's correlation	p-value
PictureA1	-	-	0.311	.026
PictureA2	0.555	.002	0.616	<.001
PictureA3	-	-	0.352	.011
PictureA4	-	-	0.308	.028
PictureA5	-	-	-	-
PictureA6	-	-	-	-
PictureA7	0.456	.013	0.421	.002
PictureA8	-	-	0.411	.003
PictureA9	-	-	0.343	.015

Table VI. Correlation analysis (Spearman's method) for differential B and differential C within the experimental and control groups

Picture Number	Group 1		Group 2	
	Spearman's correlation	p-value	Spearman's correlation	p-value
PictureA1	-	-	-	-
PictureA2	0.423	.022	-	-
PictureA3	0.404	.030	0.524	.015
PictureA4	0.884	<.001	0.734	<.001
PictureA5	0.422	.022	-	-
PictureA6	-	-	-	-
PictureA7	0.612	<.001	-	-
PictureA8	0.601	<.001	-	-
PictureA9	-	-	-	-

perceiving a picture is directly related to the complexity of the picture itself, then both groups of respondents react to such complexity in the same way. On the other hand, on the example of Fig. 8 A7, it can be seen that the respondents of the experimental group, on average, understood its essence better than the respondents of the control group.

A frequency analysis (Table III) of the responses from the control and experimental groups was done based on the B differential, which shows how interested the respondents were in pictures. The results showed that the respondents of the experimental and control groups considered the same pictures least interesting, but they considered different pictures the most interesting. Thus, the respondents of both groups found Fig. 8 A1 and Fig. 8 A9 the least interesting. Both groups of respondents had similar ideas about the types of the pictures that were not interesting to them. This may be due to the fact that the both Pictures (A1 and A9) had similar stylistic and content features. The respondents of the experimental group were interested in Fig. 8 A2, A5, A6 and A7.

We assumed that people in the experimental group would be more likely to agree that the pictures showed the same emotion as the author noticed than people in the control group. Such a tendency was observed for Fig. 8 A1, A4, A7, A8 and A9. On the other hand, for Pictures A3, A5 and A6, the respondents of both groups did not note the correspondence of the emotion noted by the author of the pictures. Fig. 8 A2 was evaluated by respondents without a clear expression of correspondence to a certain emotion (Table IV).

The following trend can also be seen from the above tables: the respondents of the experimental group tend to respond to the semantic differential by marking the extreme answers, i.e., "do not understand at all/completely understand", "not at all interesting/very interesting", "fully corresponds to a certain emotion or does not at all correspond to a certain emotion". On the other hand, respondents in the control group noted intermediate values more.

We suggested that the correlation between picture understanding and interest in the picture would differ between the control and experimental groups. For this purpose, a Spearman's rank correlation coefficient was used to check whether there were any statistically significant relationships between the responses of people within each group. The results show that for the control

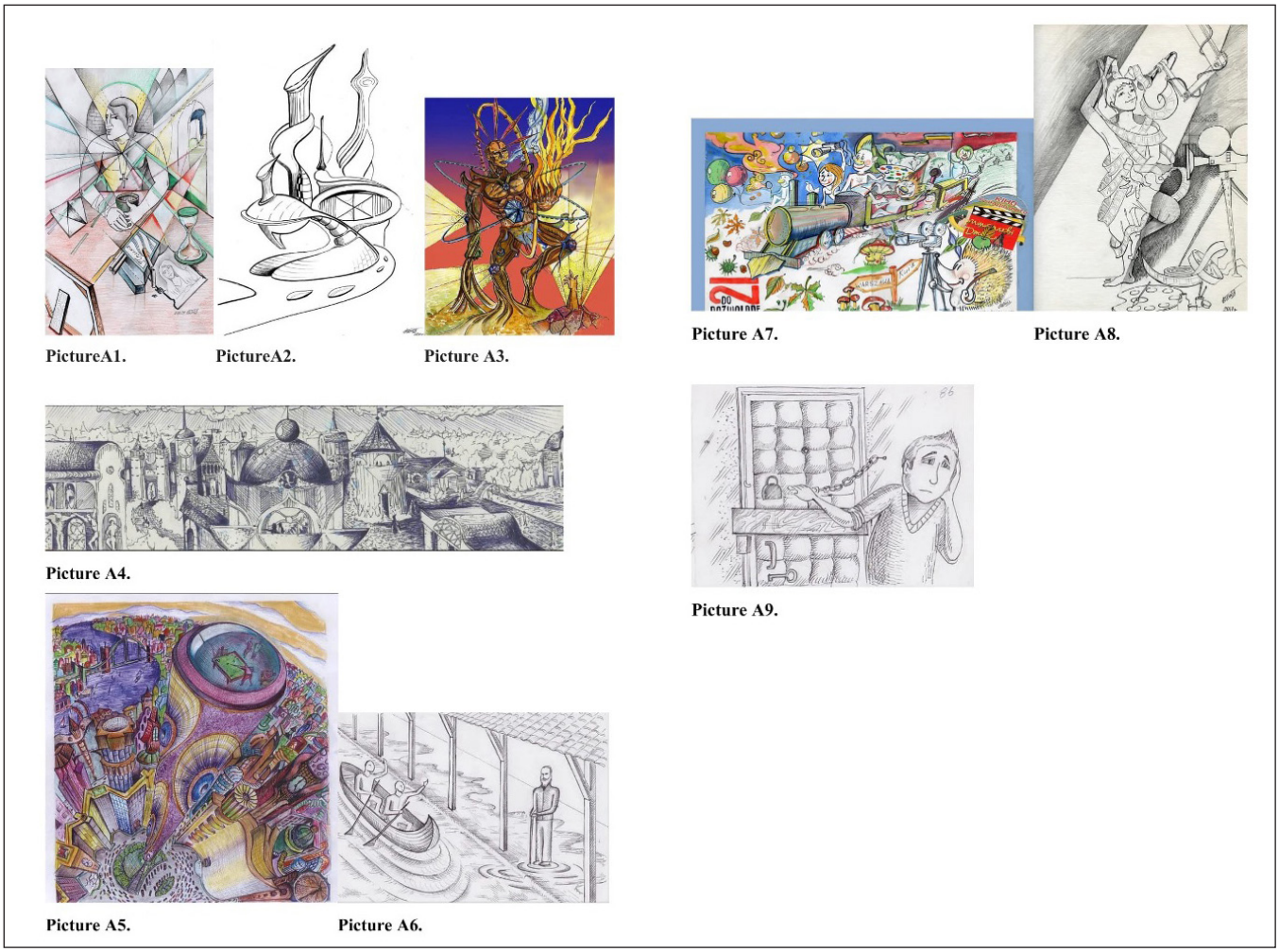

Fig. 8. Visualization of correlations for the control group (Picture A3 and Picture A4)

group, understanding the picture was more important than being interested in it, and vice versa: being interested in the picture can affect how well you understand it. A weak positive correlation was found for Fig. 8 A1, A3, A4 and A9, a moderate correlation for Fig. 8 A7 and A8, and a strong correlation for Picture A2 (Fig. 5).

For the experimental group, a moderate level of positive correlation was established between understanding the picture and interest in it in Fig. 8 A2 and A7 (Fig. 6; Table V).

Moreover, we assumed that the correlation between interest in a picture and the degree of expressiveness of a certain emotion in the same picture would differ in the control and experimental groups. For this purpose, a Spearman's rank correlation coefficient was used. The results show that in the experimental group, respondents indicated greater correspondence to a particular emotion when they expressed more interest in the picture, and vice versa. A positive correlation was established for the experimental group at a moderate level for Fig. 8 A2, A3 and A5, a significant level for Fig. 8 A7 and A8, and a strong level for Fig. 8 A8 (Fig. 7). For the control group, a moderate level of positive correlation was established for Fig. 8 A3 and a strong level for Fig. 8 A4 (Fig. 8; Table VI).

DISCUSSION

The degree of abstractness or objectivity in drawings influences the level of understanding of these depictions by both patients with schizophrenia and those with other pathologies. Analysis of the responses from schizophrenic patients revealed a superior understanding of abstract images compared to the control group (Fig. 8 A4 and A6 exhibited a high level of objectivity, while Fig. 8 A2 was the most abstract). One possible explanation for this is that the less abstract images often contain a specific situational context, which might be more challenging for patients with schizophrenia to comprehend. In other words, assembling the picture's details into a cohesive whole, or grasping the narrative within the image, might have been more difficult for them. This theory is supported by the findings of Chen et al. [26], where the authors discovered that patients

with schizophrenia showed decreased sensitivity to manipulations of visual features in artwork. This could suggest a diminished appreciation for the modulation of basic visual signals used in the arts.

Despite evidence that patients with paranoid delusions and behavioral signs may exhibit impaired understanding of abstract concepts [27], patients with schizophrenia tend to have a better comprehension of images that lack a singular semantic and compositional whole compared to patients with other diagnoses. This suggests that patients with schizophrenia might have general patterns for understanding visual stimuli and creating images. For instance, Fig. 8 A7, which lacks a unified composite whole and features highly fragmented details, was better understood by respondents with schizophrenia spectrum disorders than by those with other diagnoses. This leads us to speculate that patients with schizophrenia might be more inclined to focus their attention on details rather than on the overall composition's integrity. This assumption aligns directly with Cutting's study [28] that demonstrated that individuals with schizophrenia tend to be less concrete in their thinking compared to other psychiatric patients.

With respect to respondents' reactions to non-verbal stimuli, our results indicate that the degree of abstractness and detail in drawings affects the level of interest in the depicted subjects among individuals with schizophrenia spectrum disorders: the more realistic the objects in the drawings, the less intriguing they were. Hence, for patients with schizophrenia, the likely variability and vividness of the fantasies sparked by high-detail, abstract images were significant. In contrast, for patients with other diagnoses, there were no specific elements in the images that particularly piqued their interest. Thus, our findings suggest that for patients with schizophrenia, self-reported interest aligns with their level of understanding. This is consistent with the findings of study examining the relationship between cognition, motivation, and emotions in schizophrenia, which suggests that the motivation of these patients is primarily fueled by the so-called "appetitive" phase, that is, the phase concerning the signals indicating potential availability of the "desired" stimulus, as well as the means or behavioral tools employed to achieve this goal. It is worth noting that overall, individuals with schizophrenia are reported to have an intact visual imagery mechanism and tend to rely on visual classifications when they are ill , which enhances the opportunities for their study using non-verbal stimuli.

Our findings regarding emotional responses indicate that patients with schizophrenia more easily identify stereotypical signs of emotions compared to patients with other diagnoses. We observed a trend wherein an increase in interest in a non-verbal stimulus among patients with schizophrenia enhances their ability to judge the extent to which each picture represents a specific emotion. This aligns with the findings of , which reveal a correlation in schizophrenics between domains of thought disorder and impaired recognition of emotion. Additionally, our results suggest that when drawings contain stereotypical indicators of a certain emotion, patients with schizophrenia are typically able to recognize such emotions. Specifically, these patients demonstrated better results in interpreting emotions from facial expressions, if present, while other elements of the composition, such as lighting, colors, and background details, had little effect on their emotional response.

Existing research shows varied results regarding the emotional perception of non-verbal stimuli in patients with schizophrenia. Some studies suggest that emotion recognition in patients with schizophrenia is poorer than in healthy individuals and patients with other diagnoses. Conversely, one study indicates that patients with schizophrenia are highly sensitive to certain negative emotions, such as fear and anger. Hempel et al. investigated the physiological reactions of schizophrenic patients to emotional stimuli and found altered physiological responses to images with positive emotional content. Another study revealed that patients with schizophrenia have a poorer perception of others' negative emotions. The latter three studies support our results, and we posit that the discrepancies with the first two studies stem from differences in specific experimental setups.

In conclusion, despite the greater tendency of patients with schizophrenia to select extreme responses in semantic differentials, the self-reported responses to non-verbal stimuli demonstrate a significant difference when compared to patients with other diagnoses. Thus, our results align with studies that affirm the applicability of non-verbal stimuli in evaluating and distinguishing the presence or severity of certain aspects of schizophrenia. Specifically, these are study, which proposes that meaningful interpretation of pictures might be a valuable tool for assessing the distortion of meaning characteristic of schizophrenia; study, suggesting that non-verbal speech cues can reliably differentiate between subjects and controls; and study, asserting that non-verbal speech cues are objective measures of the negative symptoms of schizophrenia.

A significant limitation of this study was the disproportionately high number of male participants compared to females. Additionally, the difficulty or even impossibility of conducting the research with a larger patient population presents a considerable limitation.

Nevertheless, the findings of this study have potential implications for improving art therapy interventions for patients with schizophrenia. By understanding patients' preferences for abstract versus specific imagery, therapists may devise more effective strategies to engage them in the therapeutic process and encourage self-expression.

CONCLUSIONS

The investigation of non-verbal stimulus perception in schizophrenia patients reveals crucial information regarding their subjective understanding, affective responses, and levels of interest, thereby enhancing our understanding of the complex cognitive mechanisms underlying this mental disorder.

The self-assessed level of subjective understanding of the pictures among the participants with different diagnoses was assessed and showed that patients diagnosed with schizophrenia had a superior comprehension of visuals that lacked a coherent semantic and compositional whole.

The degree of interest demonstrated by respondents with different diagnoses towards the presented images was measured and revealed that patients who have disorders that fall anywhere along the spectrum of schizophrenia showed varying degrees of interest in the image depending on its level of abstraction as well as the level of detail it contained: abstract and highly detailed pictures gathered more attention of the patients with the schizophrenia disorder. In addition, patients who have schizophrenia spectrum illnesses found the picture to be less appealing when the items in it were more realistic.

The degree of expression of a specific emotion in relation to each image among the participants with different diagnoses was determined. Individuals with schizophrenia disorders were far more able to recognize the stereotypical indicators of emotions than those with other illnesses. Furthermore, facial expressions were the most crucial aspect in assessing the emotional component of the drawings.

To summarize, this study's results could assist in developing more sensitive diagnostic tools for schizophrenia spectrum disorders, which could subsequently contribute to the creation of novel techniques to enhance communication between patients with schizophrenia and their caregivers. Future research could explore the impact of cultural differences on the perception and creativity of patients with schizophrenia.

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ORIGINAL ARTICLE

ANALYSIS OF DEFECTS IN THE FORENSIC MEDICAL ASSESSMENT OF CHANGES DISCOVERED DURING THE EXAMINATION OF PERSONS WHO DIED FROM TRAUMATIC BRAIN INJURY

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ABSTRACT

The aim: To identify the main groups of expert defects that arise during the forensic assessment of changes detected during the examination of persons who died from a traumatic brain injury (TBI).

Materials and methods: A total of 102 repeated commission forensic medical examinations with changed conclusions in corpses with TBI were analyzed. Data processing and analysis were conducted using statistical methods.

Results: The examined forensic medical assessments of TBI with changed conclusions in corpses were categorized into the following groups: defects in establishing the diagnosis of TBI - 17.65±7.4%; defects in establishing the mechanism of TBI - 35.3±9.3%; defects in establishing the duration of TBI – 39.22±9.5%: sober - 20±12.4%; with alcohol intoxication - 80±12.4%. A combination of defects was found in 7.83±5.2% of cases. Defects that directly affected the experts' incorrect establishment of the diagnosis, mechanism, and duration of TBI were also identified.

Conclusions: The largest number of changed conclusions during the forensic medical examination of corpses in cases of TBI was due to the wrongly established duration of the trauma, accounting for 39.2±9.5%, with the vast majority of cases (80±12.4%) observed against the background of alcohol intoxication. The mechanism of trauma accounted for 35.3±9.3% of the changed conclusions. The main defects were incomplete collection of material for histological examination (90.2±5.8%) and incorrect interpretation of the results of histological examination (76.48±8.2%), along with the violation of the method of sectional examination (68.6±9.0%). Different groups of expert defects predominated in the cases with an incorrectly established diagnosis of TBI, duration of trauma, and mechanism.

KEY WORDS: traumatic brain injury, defects, forensic medical examination

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INTRODUCTION

Traumatic brain injury (TBI) is a significant concern in forensic medicine [1]. This study identified a total of 4,665 publications related to TBI in forensic medicine between 1972 and 2021, with an increasing trend in recent years. The prevalence of TBI in the world is difficult to estimate, as it is often underreported and varies by country and population. However, it is known to be a major public health concern and a leading cause of death and disability worldwide [2]. TBI can result from a variety of causes, including falls, motor vehicle accidents, assaults, and sports injuries, among others. Forensic evaluations of TBI are important for legal and medical purposes, including assessing the severity of injury, determining the cause of injury, and informing treatment and rehabilitation plans [1, 2].

Incidence and occurring mechanisms of TBI vary from one geographical area to another and even from one country to another [3]. In particular, in Ukraine, according to official data, more than 11,000 people die from TBI annually, of which 55% die at the pre-hospital stage and 41% at the hospital stage, which exceeds hospital mortality in developed countries by more than 1.5 times.[4] Thus, the problems of examination of persons who died from traumatic brain injury are relevant for forensic medical experts of Ukraine. Some of these injuries were sustained under alcohol intoxication. When performing a forensic medical examination of corpses with fatal TBI, in majority, the expert is faced with the question of determining the duration of TBI. At the same time, the presence of alcohol intoxication can significantly change the morphological picture of TBI and lead

to false conclusions, in particular, regarding duration of TBI. Rules for conducting corps forensic medical examination in the forensic medical examination bureau [5] regulate a clear list of material that is taken by a forensic medical expert for histological examination in cases of TBI. However, when conducting repeated commission forensic examinations in some cases, it turns out that the autopsy and collection of the necessary material is not always carried out correctly, in accordance with the established rules and in an amount insufficient to provide comprehensive answers to the issues of the resolution. For example, the lack of a set of soft tissues from the areas of damage for histological examination, especially in the presence of alcohol intoxication, in TBI limits the possibilities of reliably establishing the duration of the injury. [6] False conclusions can mislead the investigation and the court during the investigation of crimes, especially in situations where the competent authorities need to establish the time, circumstances and the person who exactly caused the injury. This becomes especially important in the presence of inconsistencies in the testimony of witnesses, the accused, etc., or in cases of repeated injuries to the victim.

In order to solve the current issue regarding the quality of conducting forensic medical examinations in cases of fatal TBI, Ukraine should analyze expert cases, establish peculiarities, regularities, and identify the main causes of defects that arise when conducting forensic medical examinations in fatal cases of TBI and their causes.

THE AIM

The aim of the study is to identify the main groups of expert defects that arise during the forensic assessment of changes detected during the examination of persons who died from a traumatic brain injury, according to the Government Specialized Institute (GSI) "Main Bureau of Forensic Medical Examination of the Ministry of Health of Ukraine" (2017-2021).

MATERIALS AND METHODS

Materials were repeated commission forensic medical examinations with changed conclusions in corpses with TBI (repeated examinations, which were appointed in cases when the results of the initial examinations caused doubts or significantly contradicted other data of the proceedings and were performed by a commission of experts with the involvement of specialists in the field of forensic histology, neurosurgery, etc.), carried out at the GSI "Maine Bureau of Forensic Medical Examination of the Ministry of Health of Ukraine" in 5 years (2017-2021). 102 examinations were selected from the total number of analyzed 250 examinations that were conducted in relation to persons who died as a result of TBI. Examinations were selected for corpses without putrefactive changes at the time of autopsy (primary forensic examination) with preserved archival histological material.

The corpses were divided according to the following criteria: gender and age of the victim, duration of injury, time of injury, the circumstances of the case, diagnosis at the initial forensic examination, mechanism, presence/ absence of alcohol intoxication. The quality and completeness of forensic examination was also taken into account: autopsy, description of macro- and microscopic changes.

The following statistical indicators were determined: arithmetic mean values ($M\pm\sigma$), where σ is the root mean square deviation, frequency of cases (N) and ($P\pm95\%CI$), where 95%CI is a 95% confidence interval. Determination of the reliability of differences in proportions was carried out according to the Student's test for relative values (t). The significance level was $p<0.05$.

RESULTS

In the course of the study, 250 conclusions of forensic medical examinations of persons who died from TBI carried out during the last 5 years (2017-2021) at the GSI "Main Bureau of Forensic Medical Examination of the Ministry of Health of Ukraine" were studied. When conducting an analysis of the compliance and reasonableness of the results of the primary examinations, three groups were obtained, namely: in 102 cases, the conclusions of the primary examinations were changed completely or significantly during the repeated commission examinations, in 66 cases, the conclusions of the primary examinations were clarified, and in 82 cases, the conclusions were fully confirmed.

For further research, cases were selected in which the conclusions of the initial examinations were changed completely or in a significant way. When conducting a retrospective analysis of these 102 forensic medical commission examinations, the following research results were obtained.

The average age of those who died from TBI was 45.25±12.5 years. Among the deceased, 72 (70.6%) were male, and 30 (29.4%) were female. The average age of male deceased individuals was 43.25±11.6 years, while for female deceased individuals, it was 47.65±12.3 years.

Out of all the cases studied, 16 (15.7%) corpses were delivered from medical institutions, while the remaining 86 (84.3%) cases were corpses delivered from the scene. In all the studied cases, there was a severe acute TBI.

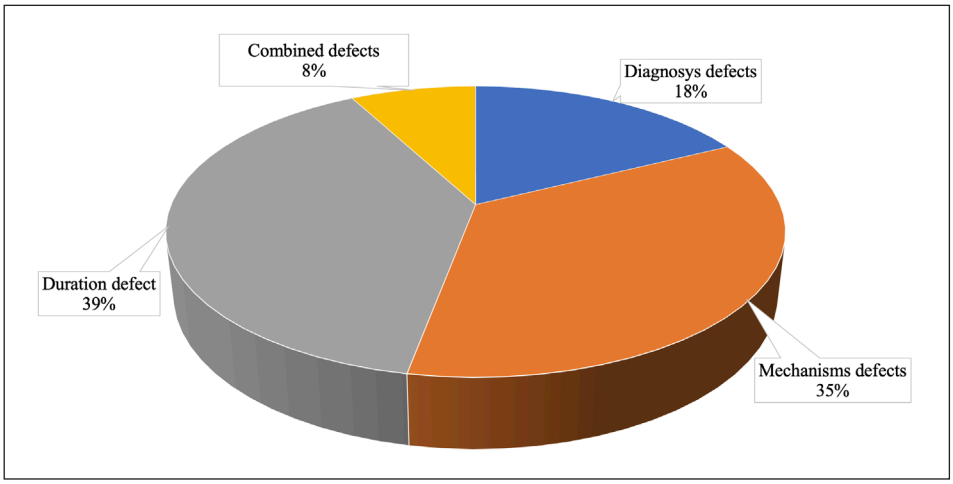

Fig. 1. Groups of defects that arise during foresic medical examinations of TBI

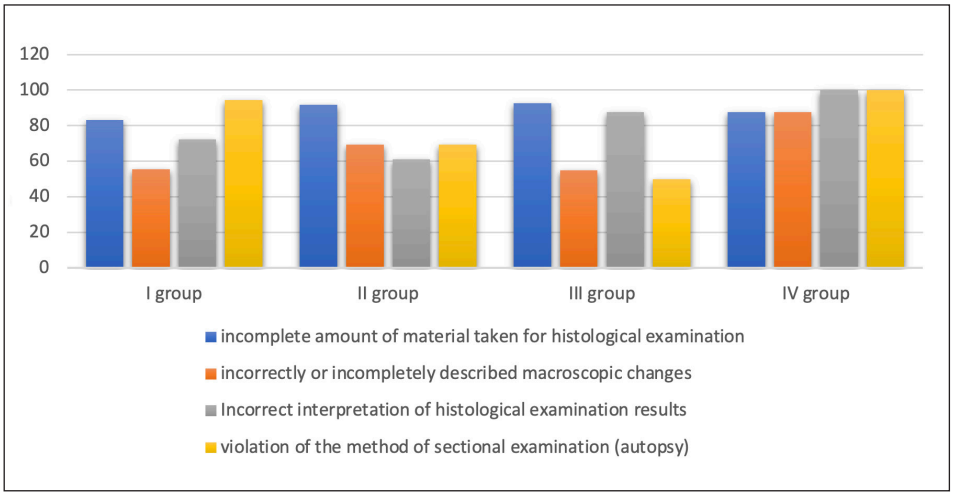

Fig. 2. Distribution of expert defects in the main groups of changed expert conclusions

Table I. Characteristics of the object of study

Indicator	Gradation	N, abs.	P, %	95%CI, %
Deaths by gender	Male	72	70,6	8,8
	Female	30	29,4	8,8
The place from which the corpse was taken to the morgue	From the scene	86	84,3	7,1
	From a medical institution	16	15,7	7,1
Presence of alcohol in blood	Was not	22	21,6	8,0
	Was	80	78,4	8,0
Amount of material taken for histological examination	Right	10	9,8	5,8
	Wrong	92	90,2	5,8
Description of macroscopic changes	Wrong	64	62,7	9,4
	Right	38	37,3	9,4
Interpretation of histological examination results	Wrong	78	76,5	8,2
	Right	24	23,5	8,2
Method of sectional research (autopsy)	Right	32	31,4	9,0
	Wrong	70	68,6	9,0

The presence of alcohol in the blood of the corpses was established in 80 (78.4%) cases, and in 22 (21.6%) cases, alcohol was absent.

We categorized all the examined forensic medical examinations of persons who died from TBI with changed conclusions into the following groups:

I. Defects in establishing the diagnosis of TBI - 17.65±7.4% (18 cases).
II. Defects in establishing the mechanism of TBI - 35.3±9.3% (36 cases).
III. Defects in establishing the duration of TBI – 39.22±9.5% (40 cases), which were further divided into:
1. Cases of sober persons - 20±12.4% (8 cases).
2. Cases of persons with alcohol intoxication - 80±12.4% (32 cases).

Combination of defects - 7.83±5.2% (8 cases).

IV. Groups of defects that arise during foresic medical examinations of TBI (Fig. 1).

During a comprehensive analysis of each of the groups, the following defects were identified, which directly affected the incorrect establishment of the diagnosis, mechanism, and history of TBI:

1. Violation of the method of sectional examination (autopsy) (incomplete or poorly performed dissection, which does not comply with the Rules for conducting the corpse forensic medical examination in the forensic medical examination bureau) was detected in 68.62±9.0% (70 cases).
2. Incorrectly or incompletely described macroscopic changes (damage to soft tissues of the head, skull bones, meninges, brain matter, etc.) were found in 62.74±9.4% (64 cases).
3. An insufficient amount of material taken for histological examination was found in 90.2±5.8% (92 cases).
4. Incorrect interpretation of histological examination results was found in 76.5±8.2% (78 cases), out of which 56 cases were associated with alcohol intoxication (Table I). We will indicate the share of this or that defect in the IV group, but we will not compare it with the monodefect group.

The presence of alcohol in the blood was ascertained in corpses from group I (defects in establishing the diagnosis of TBI) in 77,8±19,2% (14 cases), from group II (defects in establishing the mechanism of TBI) – 72,2±14,6% (26 cases), from group III (defects in establishing the duration of TBI) – 80,0±12,4% (32 cases), from group IV (combination of defects) – 100±32,4% (8 cases). The difference in shares between the groups had not reached a statistically significant level.

The collection of material for histological examination in corpses was done incorrectly from group I (defects in establishing the diagnosis of TBI) in 83.3±17.2% (15 cases), from group II (defects in establishing the mechanism of TBI) – 91.7±9.0% (33 cases), from group III (defects in establishing the duration of TBI) – 92.5±8.2% (37 cases), and from group IV (combination of defects) – 87.5±22.9% (7 cases). The difference in shares between the groups had not reached a statistically significant level.

Incorrectly or incompletely described macroscopic changes were found in corpses from group I (defects in establishing the diagnosis of TBI) in 55.6±23.0% (10 cases), from group II (defects in establishing the mechanism of TBI) – 69.4±15.0% (25 cases), from group III (defects in establishing the duration of TBI) – 55.0±15.4% (22 cases), and from group IV (combination of defects) – 87.5±22.9% (7 cases). The difference in shares between the groups had not reached a statistically significant level.

Incorrect interpretation of the results of histological examination was found in corpses from group I (defects in establishing the diagnosis of TBI) in 72.2±20.7% (13 cases), from group II (defects in establishing the mechanism of TBI) – 61.1±15.9% (22 cases), from group III (defects in establishing the duration of TBI) – 87.5±10.2% (35 cases), and from group IV (combination of defects) – 100±32.4% (8 cases). The share of incorrect interpretation in group III was significantly greater at a statistically significant level ($p<0.05$) compared to group II.

Violation of the method of sectional examination (autopsy) was detected in corpses from group I (defects in establishing the diagnosis of TBI) in 94.4±10.6% (17 cases), from group II (defects in establishing the mechanism of TBI) – 69.4±15.0% (25 cases), from group III (defects in establishing the duration of TBI) – 50.0±15.5% (20 cases), and from group IV (combination of defects) – 100±32.4% (8 cases). Violation of the method of sectional examination (autopsy) significantly influenced the establishment of the diagnosis of TBI compared to the other groups ($p<0.05$) (Fig. 2).

DISCUSSION

The results of the present study on the factors influencing the accuracy of forensic medical examinations of TBI are consistent with some of the findings reported by other researchers.

The present study examined the errors in forensic assessment of TBI), and the results showed that defects in establishing the duration of TBI were the most common, especially in cases involving alcohol intoxication. In comparison, the study by Alexis et al. [8] focused on the clinical and autopsy findings in fatal head injuries. Although the two studies differ in their main focus, they share a common theme of highlighting errors in forensic assessment related to head injuries.

Interestingly, both studies identified errors related to incomplete or inadequate autopsy examination [7,8]. Scendoni et al. [7] reported that a violation of the method of sectional examination was detected in more than two-thirds of the cases, which is a crucial step in the autopsy process to detect and describe

macroscopic changes. Similarly, Alexis et al. [8] found that inadequate autopsy examination led to missing or incomplete description of macroscopic changes.

Another common theme between the studies is the presence of alcohol intoxication in a significant proportion of cases. Scendoni et al. [7] reported that alcohol was present in the blood of the deceased in almost 80% of cases, which was associated with a higher proportion of errors in establishing the duration of TBI. Alexis et al. [8] also found that alcohol intoxication was a significant factor in fatal head injuries, as it was present in more than half of the cases.

Bertozzi et al. [9] conducted a literature review on TBI from a forensic approach, and identified factors that could influence the accuracy of TBI diagnosis, including incomplete or inadequate examinations, misinterpretation of findings, and inadequate documentation. These findings are consistent with the present study's findings regarding violations of the method of sectional examination.

Overall, these studies suggest that the accuracy of forensic medical examinations of TBI can be influenced by a range of factors, including incomplete or inadequate examinations, misinterpretation of findings, and inadequate documentation. Therefore, efforts should be made to improve the quality of forensic medical examinations through appropriate training and standardization of protocols.

CONCLUSIONS

1. The largest number of changed conclusions during the forensic medical examination of corpses in cases of TBI was due to the wrongly established duration of the trauma, accounting for 39.2±9.5%, with the vast majority of cases (80±12.4%) being observed in individuals with alcohol intoxication. The mechanism of trauma accounted for 35.3±9.3% of the changed conclusions.
2. Defects that directly affected the incorrect establishment of the diagnosis, mechanism, and duration of TBI by experts were the incomplete collection of material for histological examination (90.2±5.8%) and the incorrect interpretation of the results of histological examination (76.48±8.2%). Additionally, the violation of the method of sectional examination (68.6±9.0%) was also a contributing factor.
3. Violation of the method of sectional examination (94.4±10.6%) had a statistically significantly greater ($p<0.05$) influence on the diagnosis of TBI. On the other hand, incorrect interpretation of the results of histological examination had the most statistically significant effect on the determination of the history of TBI, with defects in this group accounting for 87.5±10.2%. In examinations with a combination of defects, the mechanism of their appearance did not depend on the type of defect, as they were present in almost all cases.
4. The nature of the defects established during the specified analysis indicates the need for:

a. Organization of additional courses with an in-depth study of relevant material and improvement of sectional techniques for experts.
b. An accurate forensic approach and strict compliance with the Rules for conducting corpse forensic medical examinations in the forensic medical examination bureau and the scope of research.
c. Implementation and improvement of a quality control system in forensic medical examination institutions.
d. Organizational and procedural changes to regulate the workload of forensic medical experts, as well as improving working conditions and expanding the capabilities of forensic laboratories.

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ORIGINAL ARTICLE

VITAMIN D STATUS IN CHILDREN WITH PARALITIC SYNDROMS

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ABSTRACT

The aim: Determination of serum 25(OH)D in the children with paralytic syndromes and its distribution depending on age, sex, taking anticonvulsant drugs, nutritional status for a period of one year (autumn-spring) of one center.

Materials and methods: There were recruited of 77 children with paralytic syndromes and 73 health children for the same period aged from 1 till 18 years. The study included a scrutiny of medical history and analysis of medical documents, assessment of motor dysfunction by GMFCS, and nutritional status.

Results: Among children with paralytic syndromes there were spastic tetraparesis 59.7%, malnutrition 92%, IV-V level of gross motor disfunction 80.5%, antiseizure medications 59.7% and cognitive impairment 77.9%. The variation of serum 25(OH)D is from 6.1 to 76.7 ng/mL with median 18.3 ng/mL in healthy children. The variation of serum 25(OH)D is from 2.2 to 83.0 ng/mL with median 14.8 ng/mL in children with paralytic syndromes (p=0.0103). Vitamin status among them is the following: insufficiency (21–29 ng/mL)–28.7% vs 16.8%; deficiency (<20 ng/mL)–56.1 vs 72.2% (p=0.0300). The 25.9% children with paralytic syndromes and those who have deficiency demonstrate severe deficiency (<10 ng/mL) compare 10.9% in healthy children (p=0.00189). There is a tendency to decrease of serum 25(OH)D in children with paralytic syndrome older 7 years.

Conclusions: We failed to record a significant difference in the 25(OH)D between males and females, between different level of GMFCS, and anticonvulsants using. Deficiency of vitamin D in 2.25 times higher in children with paralytic syndromes and severe malnutrition. Additional researches with specific items are need in perspective.

KEY WORDS: children, paralytic syndromes, vitamin D deficiency, COVID-19 pandemic

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INTRODUCTION

The thesis that millions of children may have suboptimal levels of vitamin D is formulated was confirmed in one national epidemiologic study of serum 25-hydroxyvitamin D (25(OH)D) in the USA pediatric population (ages 1-11 years): vitamin D deficiency is about 15%, and severe deficit up to 2% of the population [1]

Regarding the European population, the results of a meta-analysis of studies including 55,844 total participants, including 14,971 children (1–18 years), showed that 13 % European individuals had serum 25(OH)D < 30 nmol/L on average in the year. Strategies should therefore be developed to ensure vitamin D intakes that are protective against its deficiency in the majority of the European population. The prevalence of vitamin D deficiency and insufficiency varies significantly both in different countries and in different subpopulations of children depending on age and comorbidities [2].

Vitamin D is a hormone with a pleiotropic effect on many organs and tissues, and its deficiency plays role in the pathological mechanisms of many diseases. Among the pediatric population, low concentrations of serum 25(OH)D are associated with hypertension, obesity, metabolic syndrome, upper respiratory tract infections, vasculitis, chronic kidney disease etc. [3 - 8].

In 2011, Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline was published [9]. Firstly, the recommendations define Vitamin D deficiency is defined as a 25(OH)D below 20 ng/ml (50 nmol/liter), and vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml (525–725 nmol/liter). Secondly, determination of 25(OH)D in serum to evaluate vitamin D status in patients who are at risk for vitamin D deficiency. Conditions such as impaired motor function due to paralytic syndromes (cerebral palsy or other) are not identified in this document as indications for 25(OH)D measurement (candidates for screening). The document defines a group for screening - people who use antiseizure medications, which is possible in people with paralytic syndromes.

However, in 2017 European Society for Pediatric Gastroenterology, Hepatology and Nutrition Guidelines for the Evaluation and Treatment of Gastrointestinal and Nutritional Complications in Children with Neurological Impairment recommend the mandatory determination of laboratory parameters for assessing the nutritional

status of children with neurological impairments, which include the study of serum 25(OH)D [10].

Since there is no clear definition of the risk group for developing vitamin D deficiency in children with paralytic syndromes and strong recommendations for screening of serum 25(OH)D in this group, we would like to contribute to clarifying this issue.

Hypothesis: in children with paralytic syndromes, the frequency of insufficiency and deficiency of vitamin D is higher than in healthy children.

THE AIM

Determination of 25(OH)D in blood serum in children with paralytic syndromes. Subobjectives: 1) compare serum 25(OH)D in children with paralytic syndromes and healthy children; 2) determination of serum 25(OH)D in children with paralytic syndromes and its distribution depending on age, sex, taking anticonvulsant drugs, nutritional status.

MATERIALS AND METHODS

Study design and setting. This study is single-centered (Rehabilitation Center with out-patient service), retrospective, case-control type. This study was performed for a period from October 2021 till March 2022 (autumn – spring seasons). We evaluated demographic and clinical data, determined the motor dysfunction, and assessed nutritional insufficiency.

Ethical approval. This study was approved by the Ethics Committee (The protocol № 5, date October 2021), which was conducted with the involvement of underage patients and did not contain measures that could harm their health. Both parents were informed about the methods and scope of the study and agreed to the participation of their children in this study.

Sampling. 150 children were involved in the study for one year period. The age of the children ranged from 1 to 18 years. Study group included 77 children with paralytic syndromes. Control group included 73 healthy children appropriate ages, who was recruited as volunteers from out-patient department with routine pediatrics observation and who had no any chronic and acute diseases during blood collection time.

Inclusion criteria: children 1 - 18 years with paralytic syndromes according to ICD - 10 (cerebral palsy G 80, hemiplegia G 81, paraplegia and tetraplegia G 82, other paralytic syndromes G 83) associated with CNS damage caused by hypoxia, bleeding, thrombosis, trauma; congenital brain defects.

Exclusion criteria: rickets-like hereditary diseases, undiagnosed progressive conditions with disorders of the central nervous system of unclear etiology, rickets in young children, congenital or hereditary skeletal disorders, liver and kidney diseases and those who were already taking synthetic vitamin D supplements.

Data collection. The study included a detailed scrutiny of medical history and analysis of medical documents, assessment of objective child' examination, anthropometric measurements and assessment by Gomez classification of protein – energy malnutrition [11], and movement disorders levels according to the GMFCS [12].

Determination of serum 25(OH)D (ng/mL) in children was performed by enzyme immunoassay on the analyzer "Labline-90" (Austria) using a commercial test system manufactured by Diagnostics Biochem Canada Inc. (Canada) according to the instruction provided. All 150 portions of blood serum were collected with serum storage in the freezer at -20°**C** for no more than one month. According to Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline, deficiency <20 ng/mL, insufficiency - between 21 and 29 ng/mL, and sufficiency >30 ng/mL were considered to evaluate the results of serum samples for 25(OH)D [9].

Statistical analysis was performed with the program MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; 2018). Descriptive analysis, odds ratio (OR), relative risk (RR) and the 95% confidence interval (CI) was determined. For all parameters, their distribution was checked using the Shapiro-Wilk test. For comparison of two independent groups the Mann-Whitney (MW) test was used. For comparing proportions, the Chi-squared test was applied. The difference in parameters was considered statistically significant at $p < 0.05$ and if 95 % CI excluded of «1».

RESULTS

Table I presents demographic characteristics of children with paralytic syndromes and healthy children.

The distribution of children by age group was equal between children with paralytic syndromes and healthy children except young children. Among both the preschool aged children prevailed, and every fifth child with paralytic syndromes lived in rural areas. Table II presents clinical data of children with paralytic syndromes.

Some significant states and comorbidities were prevalent in this cohort. So, 71 (92 %, p=0.0001) children had malnutrition and 62 (80.5 %, p=0.0001) of them had IV - V level of gross motor disfunction. More than half 46 (59.7, p=0.0001) of the children had convulsions and antiseizure medications as mono- or bitherapy (leveti-

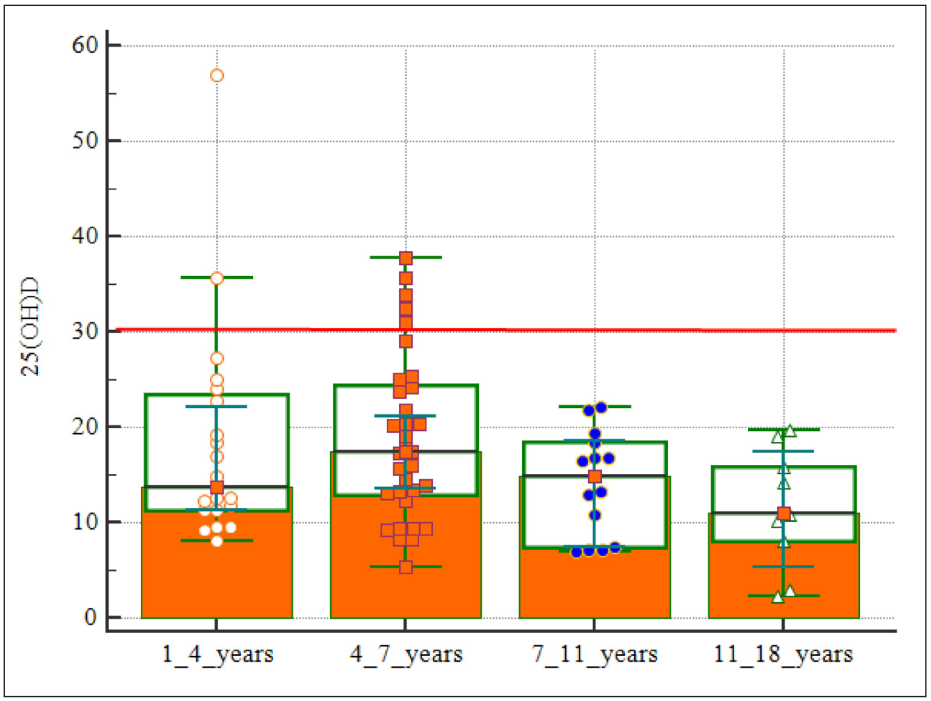

Fig. 1. Distribution of serum 25(OH)D depending on age in children with paralytic syndromes

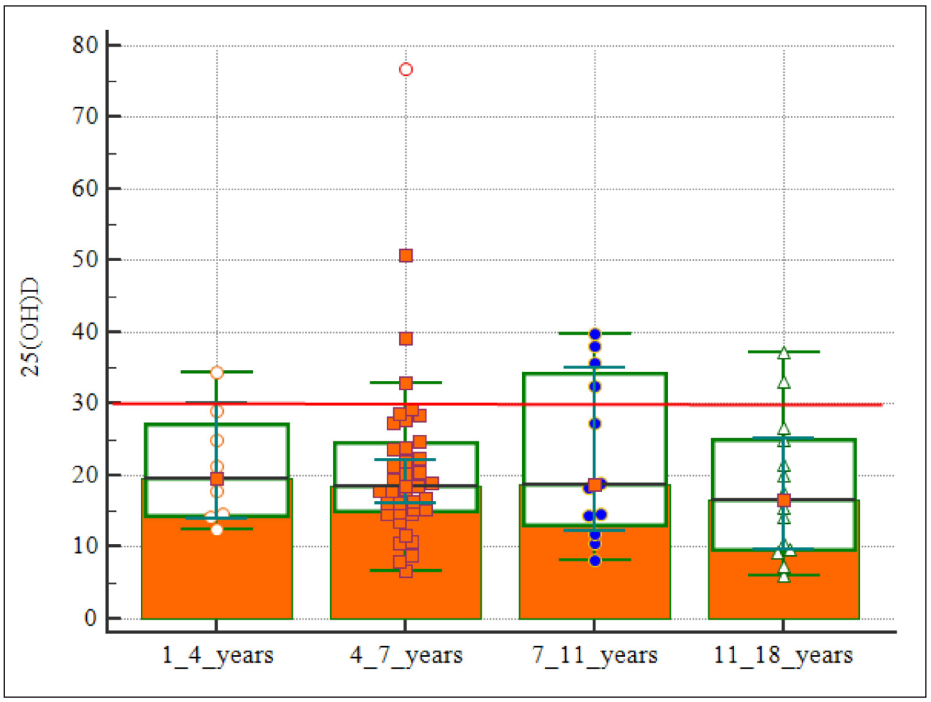

Fig. 2. Distribution of serum 25(OH)D depending on age in healthy children

racetam, carbamazepine, oxcarbazepine, clonazepam, valproic acid, gabapentin). 60 (77.9 %, p=0.0001) of the children with paralytic syndromes were mentally destroyed. Spastic tetraparesis was more common among the various types of paralytic syndromes - 50 (59.7 %, p=0.0035).

Table III demonstrates the medians, distribution and vitamin D status in total cohort.

There were significant differences between serum 25(OH)D in compare groups and a high proportion of vitamin D deficiency and severe deficiency among children with paralytic syndromes. The minimum value of 25(OH)D was 2.2 ng/mL in child with severe deficiency.

We did not obtain a significant difference when calculating the probability of two states of insufficiency and deficiency in children with paralytic syndromes compared with healthy children (OR=1.5, 95% CI 0.57 – 4.04, p=0.3915).

Another point of study was the assessment of the serum 25(OH)D in the children with paralytic syndromes depending on the sex, GMFCS level, protein – energy malnutrition, anticonvulsants using, and age.

Table I. Demographic data of children with paralytic syndromes

Data	Children with paralytic syndromes n=77	Healthy children n=73	p
Age, years Me [min; max]	5 [1.0; 17.5]	6.5 [1.0; 17.0]	0,7658*
1 – 4 years	20 (25.9)	8 (10.9)	0.0189
4 – 7 years	33 (42.8)	39 (53.4)	0.2220
7 – 11 years	14 (18.1)	12 (16.4)	0.7455
11 – 18 years	10 (12.9)	14 (19.1)	0.2368
Male abs., (%)	48 (62.4)	39 (53.4)	0.2713
Female abs., (%)	29 (37,6)	34 (46.6)	0.2713
Residents of rural areas	16 (20.7)	6 (8.2)	0.0260

*MW test

Table II. Clinical data of children with paralytic syndromes

Presentations	n (%)	95 % CI
	Category	
Spastic paraparesis	4 (5.1)	2.0 – 12.6
Spastic tetraparesis	46 (59.7)	48.5 – 69.9
Dyskinetic disorders	9 (11.7)	6.2 – 20.7
Double hemiplegia	18 (23.2)	15.3 – 33.9
	GMFCS	
II - III level	15 (19.4)	12.1 – 29.6
IV - V level	62 (80.5)	70.3 – 87.8
	Protein – energy malnutrition	
Mild	12 (15.6)	9.1 – 25.2
Moderate	6 (7.8)	3.6 – 15.9
Severe	53 (68.8)	57.8 – 78.0
	Complications and co-morbidity	
Mental retardation	60 (77.9)	67.4 – 85.7
Convulsions	46 (59.7)	48.5 – 69.9
Visual impairment	29 (37.6)	27.6 – 48.8
Skeleton deformations	16 (20.7)	13.2 – 31.1

Comparison of the serum 25(OH)D did not reveal any differences in the sex: 16.3 [min – 2.8; max – 83.0] ng/mL and 13.1 [min – 2.2; max – 33.9] (MW test, p = 0.3205) ng/mL respectively; between different level of gross motor disfunction II - III and IV - V level: 17.1 [min – 9.3; max – 56.9] ng/mL, and 14.5 [min – 2.2; max – 83.0] (MW test, p = 0.2193) ng/mL respectively; and anticonvulsant using or no: 15.7 [min – 2.2; max – 33.2] ng/mL, and 14.2 [min – 2.8; max – 83.0] (MW test, p = 0.9188) ng/mL respectively. Significant differences of the serum 25(OH)D were found in the children with paralytic syndromes who had severe malnutrition compare mild and moderate malnutrition: 13.3 [min – 2.8; max – 83.0] ng/mL, and 17.8 [min – 7.0; max – 56.9] (MW test, p = 0.0464) ng/mL respectively.

We found the interesting results on the distribution of serum 25(OH)D depending on age in children. Graphical analysis demonstrates a decrease in serum 25(OH)D in the children with paralytic syndromes, depending on the age of the child, in contrast to healthy children (Fig. 1, Fig. 2). The serum 25(OH)D less than 30 ng/mL completely presented in children with paralytic syndromes.

Summarizing the results obtained, using binary logistic regression and relative risk calculation, we identified the most significant factors associated with hypovitaminosis D. We compared of the relationship between vitamin D deficiency with some contributive factors as GMFCS I-III and IV - V level; moderate and severe malnutrition (Gomes classification), age > and < 7 years in children with paralytic syndromes. We have established a relationship of vitamin D deficiency with GMFCS IV - V level; severe malnutrition, and the age > 7 years, but only valid for the severe malnutrition (Table IV).

Table III. 25(OH)D serum levels in children and its description

Index/Presentation	Children with paralytic syndromes n=77	Healthy children n=73	p
25(OH)D, ng/mL Me [min; max]	14,8 [2.2; 83.0]	18,3 [6.1; 76.7]	0,0103*
Sufficiency (> 30 ng/mL)	8 (10.3)	11 (15.0)	0.7394
Insufficiency (21 – 29 ng/mL)	13 (16.8)	21 (28.7)	0.0812
Deficiency (< 20 ng/mL)	56 (72.7)	41 (56.1)	0.0300
Severe deficiency (<10 ng/mL)	20 (25.9)	8 (10.9)	0.0189

*MW test

Table IV. Deficiency of vitamin D in children with paralytic syndromes

Presentation	RR	95 % CI	p
GMFCS IV – V level	1.0	0.6 – 1.7	0.9618
Severe malnutrition (Gomes classification)	2.25	1.1 – 5.0	0.0462
Age > 7 years	1.2	0.8 – 1.8	0.3223

DISCUSSION

In this single-centered case-control study, we report the serum 25(OH)D level and vitamin D status for one-year period (autumn - spring) in children with paralytic syndromes and in the healthy children attended our Center. Our intention here was to clarify the issue for the clinical approach and of serum 25(OH)D screening to determine vitamin D status in children with paralytic syndromes. We put forward the hypothesis that the children with paralytic syndromes had high frequency of insufficiency and deficiency of vitamin D than the healthy children. The selected group for the study as children with paralytic syndromes is justified by the fact that they have many predisposing factors for vitamin D deficiency. However, there is still no consensus for this category of children regarding screening and robust recommendations for vitamin D supplementation [9, 10, 13].

We have showed the median level of serum 25(OH)D was 14,8 ng/mL and vitamin D deficiency was in 72.7 %, and insufficiency in 16.8 % in the children with paralytic syndromes.

However, we have received unexpected for us data on the high incidence of vitamin D deficiency and insufficiency in healthy children: 56.1 % and 28.7 % respectively. The finding by our point of view, of a high frequency of vitamin D deficiency among healthy children is associated with the diversity of the frequency of this status among different countries and populations. We found different published data for vitamin D status in populations in literature. As we stated above, 15 % of children aged 1-11 years in the US population are in vitamin D deficiency and 13 % European population (children and adults) [1, 2]. At the same time, a later publication states the following rates of Vitamin D deficiency: in <20% of the population in Northern Europe, in 30-60% in Western, Southern and Eastern Europe and up to 80% in Middle East countries. Severe deficiency was found in >10% of Europeans [14]. A certain period of time has passed between these publications. And we do not exclude the contribution to the development of vitamin D deficiency of such factors as less sun exposure of children and the digitalization of society, as well as the COVID-19 pandemic and lockdown restrictions [15, 16]. Our study was conducted during the period of partial lockdown measures. Moreover, the quality of life in children with CP is severely affected due to limitations in daily life activities, for instance eating, drinking, bathing, dressing, limited range of mobility owing to erratic muscle tone, wobbly gait, uncontrolled movements, poor balance, and deprived social functioning [17]. A national study in France of thousands of disabled children from birth to 18 years old studied their quality of life during the quarantine due to COVID-19. Children with an average age of 9.5 years mostly had cerebral palsy (42%) or neuromuscular diseases (11%). Quarantines negatively affected morale (44% of children), behavior (55% of children) and social interactions (55% did not have contact with other children). A total of 44% of children stopped physical activity; 76% studied at home; only 22% continued medical observation [18]. Data on the positive effects of vitamin D for the prevention or treatment of COVID-19 in adults have been published, but it is still unknown whether vitamin D deficiency increases the risk of SARS-CoV-2 infection in children [19, 20]. No randomized trials have been conducted in the pediatric population.

We analyzed the clinical features and possible contributing factors of vitamin D deficiency in children with paralytic syndromes. The cohort of children was

characterized by a high incidence of spastic tetraparesis 46 (59.7%), cognitive dysfunction 60 (77.9%), severe motor dysfunction 62 (80.5%), using of anticonvulsant therapy 46 (59.7%), and severe malnutrition 53 (68.8%). The serum 25(OH)D was lower compared with healthy children, and deficiency and especially severe deficiency were higher than in the healthy children - 72.7 % vs 56.1 % and 25.9 % vs 10.9 %, respectively. Researchers have demonstrated different frequency of vitamin D deficiency and insufficiency in children with paralytic syndromes. So in one publication Vitamin D deficiency was present in 53.4% of children with cerebral palsy versus 19.9% in those without it [21]. At the same time, according to cross-sectional study, there were 47.8 % insufficiency and 30.4 % deficiency of vitamin D in children with cerebral palsy aged 2 to 21 years. The mean value of 25(OH)D was 24.3±8.8 ng/ml and all children had moderate and severe protein – energy insufficiency. Authors advocate the control of 25(OH)D levels and vitamin D medicines should be as equipped in rehabilitation and tertiary medical centers [22]. But studies were different in design and simples.

We failed to record a significant difference in the 25(OH)D between males and females as demonstrated by most studies.

The factor reported as a risk for 25(OH)D deficiency hepatic metabolism-inducing antiepileptic drugs through cytochrome P450. According to a systematic review and meta-analysis by found that pediatric patients on cytochrome P450 enzyme inducing antiepileptic drugs had statistically significant prevalence of vitamin D deficiency (OR 0.33, 95% CI 0.21–0.47). The antiepileptic drugs were categorized according to their effect on the cytochrome P450 system as inducers (carbamazepine, phenobarbital, phenytoin, topiramate, oxcarbazepine, and primidone), non-inducers (valproic acid and clobazam), or not metabolized by this system (levetiracetam, gabapentin, ethosuximide, vigabatrin, zonisamide) [23]. The authors noted the prevalence of vitamin D deficiency in 32% among them. However, the results of this review were based exclusively on children with epilepsy without motor dysfunction, excluding timing of anticonvulsant medications using.

The next special issue for discussion is the relation between vitamin D status and different level of the motor dysfunction. We did not get significant differences in vitamin D status when comparing children with levels II – III and IV -V by GMFCS. We found different published data in this research point. So, a significant correlation between 25(OH)D and GMFCS levels and related disorders such as epilepsy, mental retardation, dental problems and retention growth was demonstrated on a sample of 274 children aged 1-19 years old with cerebral palsy and a predominance of spastic tetraparesis (59.8%) [24]. Our research confirms the published result N Paker et al. [25]. We did not find a significant relationship between vitamin D deficiency and GMFCS IV – V levels. However, we have shown a trend towards a decrease in serum 25(OH)D in children with paralytic syndromes in children older than 7 years. Similar to our study, 119 children with paralytic syndromes, among whom spastic tetraparesis was in 88.3% with average IV level of GMFCS, no statistically significant correlation was found between vitamin D levels and gender, GMFCS, type of paralytic syndrome, season of the year and treatment with antiepileptic drugs. However, sufficient serum vitamin D levels were statistically significant in children with a mean age of 3.8 years, while children with a mean age of 6.4 years had low vitamin D levels [25].

Paralytic syndromes associated with the possibility of assimilation of a sufficient number of nutritional components due to impaired chewing, swallowing, motor disorders of the gastro-intestinal tract, etc. [10]. We found that vitamin D deficiency in children with paralytic syndromes occurs with severe malnutrition by 2.25 times (RR= 2.25, 95 % CI 1.1 – 5.0, p=0.0462). Nutritional assessment and feeding tube use, as well as clinical nutrition, should be the focus of the clinician's attention especially since are no established strong recommendations indicating which patients should receive a gastrostomy tube [26].

Considering that vitamin D deficiency or resistance is caused by one of four mechanisms: impaired vitamin D availability due to insufficient dietary intake, malabsorption disorders, or lack of sunlight (photoisomerization); violation of hydroxylation by the liver for the production of 25(OH)D; violation of hydroxylation by the kidneys with the formation of 1,25(OH)2D, and insensitivity of organs to vitamin D metabolites, we believe that monitoring and timely prevention of nutritional deficiency in children with paralytic syndromes is absolutely necessary for its prevention. Undoubtedly, questions arise regarding vitamin D supplementation for children with paralytic syndromes who receive balanced clinical nutrition.

The strength of our study includes, we showed a higher incidence of severe deficiency in children with paralytic syndromes compared with healthy children, a decrease in serum 25(OH)D levels in children over 7 years of age, and a significant association with nutritional deficiencies. There were some inherent limitations associated with this study, firstly, the sample size. Our model was based on a retrospective single-center case-control study and was limited by time and number of patients. Secondly, there were very

few preliminary studies and research gaps regarding serum 25(OH)D in children with paralytic syndromes, and especially depending on the types of paralysis, which influenced the methodology of our study. Our study was limited to one autumn - spring season and took place during the COVID-19 pandemic. We were unable to assess whether there was an effect of different groups of anticonvulsants and of combined use in children with paralytic syndromes on serum 25(OH)D levels, which may undermine the strength of the study. A bias in the results of our general and subgroup analysis could be caused by the lack of data on the time of insolation of children and the study of the characteristics of their lifestyle. Finally, the origin and duration of vitamin D deficiency and insufficiency could not be elucidated. However, the results clearly deserve further study, increased data collection and improved quality of studies, especially across age groups with nutritional and lifestyle studies, in order to obtain more convincing data. Further studies are needed in our and other populations of children with paralytic syndromes to diagnose vitamin D insufficiency and deficiency and factors influencing them, both as predictors and as obligate markers.

CONCLUSIONS

1. The median level of serum 25(OH)D in children with paralytic syndromes is 14.8 ng/ml, that significantly lower than the median level of healthy children 18.3 ng/ml.
2. The vitamin status among them is as follows: deficiency (21 - 29 ng/ml) - 16.8%; deficit (<20 ng/ml) – 72.2%. 25.9% of children with paralytic syndromes and deficiency have severe deficiency (<10 ng/ml).
3. There were no any significant differences of the serum 25(OH)D in the sex, and level of motor dysfunction, and anticonvulsant using. But was a tendency to decrease of serum 25(OH)D in children with paralytic syndrome older 7 years. In this age category the serum 25(OH)D was no more than 30 ng / ml.
4. Significant differences of the serum 25(OH)D were found in the children with paralytic syndromes who had severe malnutrition 13.3 ng/mL compare mild and moderate malnutrition 17.8 ng/ml.

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ORIGINAL ARTICLE

MINI-INVASIVE TREATMENT METHODS OF SPIDER VEINS: SCLEROTHERAPY AND RADIOFREQUENCY THERMOCOAGULATION

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ABSTRACT

The aim: This study was conducted to compare the results of spider vein: sclerotherapy or radiofrequency thermocoagulation.

Materials and methods: The study included 52 patients with spider veins, who were randomized into two treatment groups: sclerotherapy or radiofrequency thermocoagulation. Treatment outcomes were assessed using: a self-assessed questionnaire, CIVIQ 20 questionnaire, computer evaluation of images, registration relapses complications, negative manifestations, and intensity of the pain syndrome.

Results: Both methods showed a statistically significant difference in the quality of life indicators before and one month after treatment ($p<0.001$ for both groups). Radiofrequency thermocoagulation showed a greater impact on the patient's quality of life ($p = 0.003$). The average length of spider veins in the treatment area decreased the most with radiofrequency thermocoagulation (by 92.1%), slightly less after sclerotherapy (by 73.4%) ($p < 0,01$).

Conclusions: Both treatments have shown good results for spider veins and were reasonably safe with few negative manifestations. Radiofrequency coagulation better eliminates small veins, less than 0.3 mm.

KEY WORDS: sclerotherapy, radiofrequency thermocoagulation, telangiectasias, spider veins, venous disorders

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INTRODUCTION

Today, a lot of attention is paid to aesthetic problems, such can be the appearance of spider veins on the lower limbs, especially in young people. According to the American Venous Forum (2008), spider veins are found in 50% of the adult population on at least one limb. Spider veins occur in two-thirds of patients before the age of 25, and increase in incidence with age [1, 2].

The pathogenesis of the appearance of telangiectasias, or the so-called spider veins, cannot be considered completely understood today. From the surgeon's point of view, the most reasonable explanation for their occurrence is venous reflux [1, 3]. Most cutaneous spider veins are abnormalities of the horizontal vascular skin plexus or capillary loops. Spider leg veins are composed of a feeder vessel and ectatic venous sprouts in the reticular dermis [2]. However, there are other points of view: hormonal changes, increased intra-abdominal pressure, hereditary features of the structure of venous vessels [4, 5].

Due to the minimal changes in the venous system, patients who are concerned about the specified cosmetic defects really agree only to the least traumatic treatment methods and most often hope for a positive result from percutaneous laser ablation. It is known that telangiectasias on the face are very well amenable to such treatment, but when they are localized on the limbs, the effect is expensive and questionable [2, 4]. Today, the gold standard in the treatment of spider veins, according to most scientists, is sclerotherapy, as it combines such positive features as minimal invasiveness, high cosmesis, lack of surgical risk, a short period of rehabilitation and does not require hospitalization of the patient [4, 6-8]. For more than twenty years of using this method as the main method of treating telangiectasia, researchers have noted a number of its complications and negative side effects. Complications are primarily caused by the introduction of a foreign substance into the body - a sclerosant, which can cause local and general reactions. Complications of sclerotherapy are divided into minor (local) and severe (systemic). Systemic complications of sclerotherapy in the treatment of spider veins are extremely rare (0.01%). These are predominantly anaphylactic reactions.

Among the local complications, pain at the injection site, local swelling, erythema, blisters, hemorrhages, local skin necrosis, and residual hyperpigmentation are the most common. According to various researches, the frequency of local complications ranges from 7% to 30% of cases [1, 3, 6, 7, 9]. Many sclerosant agents have been used but a perfect sclerosant that is complication free and 100% effective has not yet been developed. All sclerosants represent a compromise between efficacy and toxicity [2, 10].

The main disadvantage of sclerotherapy is the high frequency of relapses. Mandatory wearing of compression stockings after a treatment session causes a negative reaction of the patient and complicates the procedure in the summer season. It is technically impossible to sclerose vessels with a diameter of less than 0.3 mm because they are smaller than the diameter of the thinnest needle [3, 4, 9]. This impairs the cosmetic effect, especially in patients with a very extensive network of telangiectasias. The described factors encourage researchers to search for alternative methods of treating spider veins [11].

Now the attention of surgeons is once again attracted by hardware, coagulation techniques. These techniques were already used in the 1990s, but were forgotten due to a number of shortcomings of the technology of that time. Modern technologies, such as radiofrequency coagulation, are safer, more controlled and devoid of those disadvantages, which allows it to be used for the treatment of spider veins. This technique consists in the coagulation of blood vessels by introducing into their lumen micro needle - tungsten electrodes with a diameter of 0.2-0.3 mm. The coagulation of the vein takes place with a current of 3 MHz. The main difference between modern coagulation techniques is what is used an insulated micro needle with beveled tip. The sheath of biocompatible material covers the entire portion of the needle except the beveled tip to prevent exposure of the shaft to adjacent tissue and to minimize collateral damage [2, 12, 13].

THE AIM

The study aimed to compare the results of spider vein treatment depending on which treatment method was used: sclerotherapy or radiofrequency thermocoagulation.

MATERIALS AND METHODS

The study was conducted in accordance with the guidelines of the institutional review board and the tenets of the Declaration of Helsinki were followed and in accordance with the recommendation of International Council for Harmonization Good Clinical Practice. Informed consent was provided by all patients in the study.

From September 2018 to October 2022, 118 consecutive patients were assessed for the eligibility.

All patients in the study underwent ultrasound examination during the initial evaluation. In addition to ultrasound, vessels were visualized in the infrared spectrum of light using the Vein Finder device. The purpose of ultrasound was to detect horizontal and vertical reflux in the system of the great saphenous vein, involvement in the pathological process of malleolar region and saphenofemoral junction, as well as the spider veins feeding vessels. The examination in infrared light complemented ultrasound to find smaller feeding vessels. Patients in whom venous reflux was detected were not included in the study, since in such a case surgical intervention is advisable.

We included 52 patients with spider veins in the CEAP/C1 phase. Sixty-six patients with great saphenous vein reflux with or without involvement of malleolar region and/or saphenofemoral junction, with feeder veins larger than 1 mm, with prior surgical interventions on the veins of the lower extremities, who had sclerotherapy or laser sessions performed to the target localization, using antithrombotic medications, with allergies, pregnant, breastfeeding, with any type of skin problems were excluded.

Remaining 52 patients were randomized to receive either sclerotherapy (Group 1) or radiofrequency thermocoagulation (Group 2). The method of treatment was chosen by randomizing all patients into two groups of 26 patients using a random number table generated in the STATISTICA 13 program.

The first group included 26 patients who underwent sclerotherapy with liquid 0.5%-1% polidocanol. During the procedure, anesthesia was not used. The procedure followed by immediate topical cooling with ice packs and compression. In the post-procedural period, this group of patients received compression therapy by using compression stockings of the II compression class for 3 weeks.

The second group included 26 patients who underwent radiofrequency thermocoagulation of spider veins using the Dr. Oppel ST-501 (Sometech, South Korea). Punctures were performed in the vein projection introducing into their lumen micro needle - tungsten electrodes with a diameter of 0.2-0.3 mm. Coagulation of the vein took place at a current of 3 MHz. No anesthesia was used during the procedure. Compression therapy in the post-procedure period was not carried out, as this treatment technology does not require it.

According to the recommendations of the European guidelines for sclerotherapy in chronic venous disorders, a self-assessed cosmetic outcome and a visual

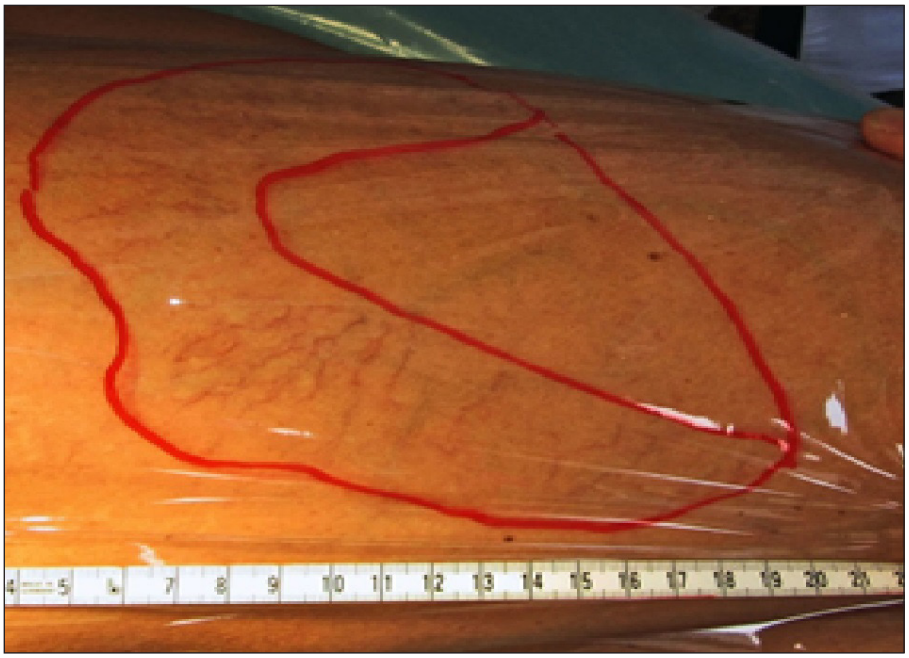

Fig. 1. Photographing the contours of the area of spider veins

Fig. 2. Segmentation of spider veins to calculate their total length in the treatment area

Fig. 3. Remains of spider veins on the periphery of the sclerosing zone one week after treatment (the zone of residual vessels is circled in red)

Table I. Patient`s self-assested cosmetic outcome

Self-assested cosmetic outcome	Group 1 n=26	Group 2 n=26	p
Better	15 (57.7%)	22 (84.6%)	0.035*
Same	10 (38.5%)	4 (15.4%)	0.062
Worse	1 (3.8%)	0	0,317

Notes: Group 1: sclerotherapy; Group 2: radiofreguency thermocoagulation.
Comparison of the data between the groups was carried out using Wilcoxon two-sample test for independent samples.
*There is significance difference

Table II. Comparison of the GIS index in patients with spider veins

	Group 1 n=26	Group 2 n=26	p
Pre- GIS	86.96±4,8	88,35±4,4	0.196
GIS 1 month after procedure	98.5±1.3	99.55±0.7	0.003*
GIS 6 month after procedure	98.31±1.4	99.23±0.9	0.007*

Notes: Group 1: sclerotherapy; Group 2: radiofreguency thermocoagulation
Comparison of the data between the groups was carried out using the Student"s test for independent samples.
*Average values differ at significance level

Table III. The total length of spider veins depending on the treatment

№	Evaluation period	The total length of spider veins			
		Group 1 n=26		Group 2 n=26	
		M ± σ (mm)	p	M ± σ (mm)	p
1	Before treatment	701,3 ± 225,8	$p_{1-2} < 0,01$*	670,2 ± 254,8	$p_{1-2} < 0,01$*
2	1 month after treatment	187,8 ± 56,79	$p_{1-3} < 0,01$*	53,01 ± 25,04	$p_{1-3} < 0,01$*
3	6 month after treatment	197,7 ± 56,26	$p_{2-3} = 0,97$	59,31 ± 27,35	$p_{2-3} = 0,99$

Notes: Group 1: sclerotherapy; Group 2: radiofreguency thermocoagulation
Comparison of the indicators in dynamics was carried out using Student"s test for related samples.
*Average values differ at significance level

Table IV. Negative manifestations and local complications encountered with various methods of treating spider veins

Complication	Group 1 n=26		Group 2 n=26		p
	n	% of patients in the group	n	% of patients in the group	
Pain at the site of the injection	6	23,1	9	34,6	0.365
Local swelling	4	15,4	0	0	0.039*
Erythema	2	7,7	2	7,7	1
Hemorrhages	1	3,8	0	0	0.317
Residual hyperpigmentation	2	7,7	0	0	0.153
In total	15	57,7	11	42,3	0.276

Notes: Group 1: sclerotherapy; Group 2: radiofreguency thermocoagulation
Comparison of the data between the groups was carried out using Wilcoxon two-sample test for independent samples.
*There is significance difference

assessment of the intervention area are sufficient to assess the treatment results [3].

In order to objectively evaluate the effect of different treatment approaches on spider veins, they were photographed with a calibrated Panasonic DMXLC15 digital camera under standard conditions (distance, lighting and no optical magnification). The contours of the area where the venous «spiders» are located

were outlined with a felt-tip pen on a transparent film applied to the skin. Bone anatomical structures were used for correct positioning of the film before and after treatment. A centimeter tape located along the zone allowed to obtain results in millimeters during computer processing of the images. (Fig. 1).

Images were analyzed using ImageJ/Fiji 1.46r J scientific image analysis software, which is available in the public domain [14]. The total length of the vascular network formed by spider veins in a certain area was calculated (Fig. 2).

The results of the treatment were evaluated at the 1st and 6th month after the treatment session. The main end point of the study was the relapse of spider veins. Relapse was considered the restoration of patency of telangiectasias in the area of therapy, as well as repeated visualization of ≥ 40%. Computer evaluation of images was performed with measurement of the total length of spider veins before and after treatment.

All complications and negative manifestations that occurred during or after the procedures were registered.

Since many patients noted unpleasant pain during the treatment, it was analyzed separately intensity of the pain syndrome on the 0–5 Numeric Rating Scale, which is often used in pain management [15].

Patients cosmetic satisfaction was also recorded on the 1st and 6th months (better/same/worse compared to the preprocedural state).

Satisfaction with treatment results was also objectively assessed using the 20-item Chronic Venous Disease quality-of-life Questionnaire (CIVIQ 20), in order to compare average scores, absolute scores were converted to a GIS index [16].

Statistical analysis was performed using Statistica 10 (Serial Number: STA999K347150-W) and MedStat. Data distribution normality was checked using the Shapiro-Uilk criterion. Normally distributed indicators were given as $M_0 \pm \sigma$, indicators whose distribution differed from the normal one, such as intensity of the pain syndrome according to the 0–5 Numeric Rating Scale, were given as M_0 (Q_1; Q_3). A comparison of the data with abnormal distribution between the groups was performed using Wilcoxon two-sample test. A comparison of the data with normal distribution was carried out using the Student"s test for independent samples, and Student"s test for related samples. The confidence intervals given in the article were constructed for the confidence level of 95%.

RESULTS

In total, only 4 patients were male, and the mean age of the study population was 39.4 ± 8.3 years. The study groups did not differ in terms of the mean age, body mass index, and the number of spider veins.

Patients' self-assessed satisfaction ratings of cosmetic outcomes were found to be higher compared to the baseline in both groups ($p < 0.001$). This cosmetic satisfaction was found to be higher in Group 2 compared to that reported by the patients in Group 1 ($p = 0.035$), (Table I).

The data obtained using the CIVIQ 20 questionnaire were also analyzed. The mean GIS of the patients before the procedure was 86.96±4,8 in Group 1 and 88,35±4,4 in Group 2. One month after the procedure, this indicator increased significantly up to 98.5±1.3 in Group 1 and 88,35±4,4 in Group 2 ($p<0.001$ for both groups). At the same time, quality of life indicators did not change significantly in the six-month period compared to the one-month period ($p = 0.30$).

The GIS comparison between Group 1 and Group 2 is shown in Table II.

Radiofrequency thermocoagulation has a greater impact on the patient's quality of life. There was a significant difference in GIS between the two groups after the procedure in both evaluation periods ($p = 0.003$ and $p = 0.007$, respectively).

Computer evaluation of images of areas with spider veins before and one month after treatment proved that both methods effectively eliminate them ($p < 0.01$ for both groups compared to the baseline). After 6 months, the total length of telangiectasias increases slightly but is not statistically significant ($p = 0.97$ and $p = 0.99$, respectively). The results are shown in Table III.

The average length of telangiectasias one month after the procedure decreased the most with radiofreguency thermocoagulation from 670,2 ± 254,8 mm to 53,01 ± 25,04 mm (by 92.1%), slightly less after sclerotherapy from 701,3 ± 225,8 mm to 187,8 ± 56,79 (by 73.4%). A statistically significant difference between the reduction in the length of the vascular network as a result of radiofreguency thermocoagulation and sclerotherapy was revealed at the level of significance $p < 0,01$. Comparison of the data between the groups was carried out using the Student"s test for independent samples.

After sclerotherapy, the distal parts of the spider veins often remained unobliterated. Additional direct puncture of them due to the small diameter of vessels (less than 0.3 mm) was practically impossible (Fig. 3). This worsened the overall cosmetic effect of sclerotherapy.

At the same time, radiofrequency thermocoagulation with a tungsten electrode with a diameter of 0.2 mm was effective even at the minimum settings of power and pulse duration (10 W, 1/8 s).

In the group of patients who underwent sclerotherapy, 3 (11.5%) relapses of the disease were registered within a month, and another 5 relapses (19.2%) within 6 months. in the group of patients who underwent thermocoagulation, 1 (3.8%) relapse was detected within a month, and 5 more (19.2%) relapses after 6 months. The difference between groups is not statistically significant (p = 0.941). Comparison of the data between the groups was carried out using Wilcoxon two-sample test for independent samples.

Analysis of the spectrum of negative manifestations and complications that occurred in patients of both groups showed that most often patients complained of pain at the injection site, and objectively, a fairly long-lasting local swelling was determined (Table IV). The difference in the frequency of complications in the examined groups is not statistically significant (p = 0.276). But in Group 1, local swelling was more common (p = 0.039).

The intensity of the pain syndrome in the Group 1 of patients who underwent sclerotherapy according to the 0–5 Numeric Rating Scale was M_0=0,31($Q_1$0; $Q_3$1), in the Group 2 M_0=0,5 ($Q_1$0; $Q_3$1). Comparison of the data between the groups was carried out using Wilcoxon two sample test. The difference between the groups was not statistically significant, (p = 0,658). However, it is worth noting that patients during thermocoagulation more often complained of more intense pain. But none of them required additional anesthesia.

DISCUSSION

There are several treatments, such as sclerotherapy, laser, intense pulsed light, microphlebectomy and thermoablation, but none is established as preferable [17].

The basic principles of treatment of varicose veins - elimination of venous reflux and removal or ablation of dilated venous vessels - have remained unchanged for a long time [1]. However, the technical means of their implementation have changed significantly over the last decade and the transformation process continues [2, 7]. This is due to the researchers' efforts to make the perioperative period or conservative treatment sessions less uncomfortable for the patient, to improve the cosmetic result of the interventions, while not increasing the risk of recurrence of varicose veins. At the same time, it is desirable not to increase the cost of treatment significantly. In our study, we tried to compare the gold standard of spider vein treatment, sclerotherapy, with the more modern and less common technique, radiofrequency thermocoagulation.

In the case of spider vein correction, success will depend on the choice of method that will provide a cosmetic result that will meet the patient's expectations to the greatest extent.

In our study, we confirmed the high efficiency of both methods in the treatment of spider veins. And we also identified some advantages of radiofrequency thermocoagulation. Almost every step of the sclerotherapy is operator-dependent from the choice of concentration of the sclerosing agent to the technique of its administration or from preparation of the field to the post-procedural follow-up [2, 6]. With radiofreguency thermocoagulation, the effect depends on the device and its settings. It can be argued that this procedure is performed under hardware control, which makes it safer. The main limitations of these methods are the reduced efficacy with increased vessel diameters and depth, and the problems experienced in selectively delivering energy to the vein during epidermal application [12].

Treatment of small vascular abnormalities of the skin is painful. In our our study, we did not find a difference in the severity of pain syndrome when using different methods. Although patients treated with thermocoagulation more often complained of pain. The sensation and severity of pain during these procedures can be explained by the fact that radiofreguency thermocoagulation refers to thermal treatment methods, so patients during the procedure note a burning sensation due to a local increase in temperature. In addition, the number of contacts of the needle with the skin during thermocoagulation is significantly greater compared to sclerotherapy.

However, it is worth noting that no patient required additional anesthesia during radiofrequency thermocoagulation. Sclerotherapy is also often accompanied by negative feelings, because a chemically active substance is injected. Patients complain of a burning sensation at the injection site, which occurs after the procedure is completed and lasts longer.

The feeling and satisfaction of cosmetic healing is not an objective parameter that can be revealed by biological tests or imaging methods. To objectively evaluate the treatment results, we analyzed the images using the publicly available scientific image analysis software ImageJ/Fiji 1.46r. In contrast to the previously described method of evaluating the surface vascularization of scars [18], the total length of the vascular network formed by spider veins in a certain area was calculated. And we have shown a significant reduction in the length of the vascular network with the use of treatment methods, which proves their effectiveness.

Spider veins are primarily an aesthetic problem. So, in the course of the study, we tried to identify the impact of the proposed methods of treatment on the quality

of life of patients using a questionnaire. CIVIQ-20 is widely used in clinical trials to evaluate post-treatment results in venous diseases [16]. In order to compare the mean scores between dimensions or scales, absolute scores were then converted into an index (GIS). According to this scoring method, improvement in quality of life between two study times is represented by an increase in score. We observed that these GIS in both groups progressed compared to the baseline values independent of the techniques used. However, our results also allow us to state that, radiofrequency thermocoagulation has a greater impact on the patient's quality of life. This may be due to the fact that thermocoagulation gives a faster result. Patients see their veins disappear during the procedure for good, while with sclerotherapy, the veins disappear during the procedure, but in a short time they become brighter, inflamed and filled with microclots. Also, thermocoagulation gives cleaner results, removing even the thinnest vessels [2, 3, 12].

As the results of the study demonstrate, none of the approaches has absolute advantages over the other. The percentage of complications and unwanted effects does not differ significantly in the three groups. We consider it expedient not to contrast them with each other, but to consider the possibility of combining in order to use the advantages of each of them.

Future studies may involve a larger population and have a longer follow-up period. And it is also possible to study the results of treatment with a simultaneous combination of these methods, or the addition of sclerotherapy with radiofrequency thermocoagulation in the long-term period in patients with poor cosmetic satisfaction. The study can be supplemented by a histological analysis of changes in the spider vein when using these methods and their combination.

CONCLUSIONS

1. Patients' self-assessed satisfaction ratings of cosmetic outcomes were found to be higher compared to the baseline ($p = 0.001$). Cosmetic satisfaction was found to be higher in in the group of patients who were treated with radiofrequency thermocoagulation compared to that reported by the patients in group with sclerotherapy ($p = 0.035$).
2. Both methods showed a statistically significant difference in quality of life indicators before and one month after treatment ($p<0.001$ for both groups). At the same time, CIVIQ 20 indicators did not change significantly in the six-month period compared to the one-month period ($p = 0.30$). Radiofrequency thermocoagulation showed a greater impact on the patient's quality of life. There was a significant difference in GIS between the two groups after the procedure in both evaluation periods ($p = 0.003$ and $p = 0.007$, respectively).
3. The average length of spider veins in the treatment area decreased the most with radiofreguency thermocoagulation (by 92.1%), slightly less after sclerotherapy (by 73.4%) ($p < 0,01$).
4. There was no significant difference in relapses and and frequency of complications between the two groups after the procedure ($p = 0.941$ and $p = 0.276$, respectively), but the local swelling was more common after sclerotherapy ($p = 0.039$). There was also no statistically significant difference in the intensity of the pain syndrome ($p = 0,658$).

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ORIGINAL ARTICLE

COMPARATIVE STUDY OF BIOTOLERANCE CHARACTERISTICS OF DIFFERENT GELS COMPOSED OF BENZYDAMINE AND FLAVONOIDS THAT WERE DEVELOPED FOR TREATMENT OF PERIODONTAL DISEASES IN ORTHODONTIC PATIENTS

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ABSTRACT

The aim: Different gels composed of benzydamine and flavonoids that were developed for treatment of periodontal diseases in the orthodontic patients will be compared regarding their effects on survival of mammalian cells of various tissue origin and their DNA intactness.

Materials and methods: Effect of different variants of patented gel composition «Benzidaflaziverdine» including a gel base and «Proteflazid®» containing flavonoids and benzydamine hydrochloride in powder form («T-Sept®») towards survival (MTT) of murine BALB-3T3 fibroblasts, J774.2 macrophages, human HaCaT keratinocytes was studied. Their effect on nativity of DNA of J774.2 macrophages was evaluated using DNA-comet assay.

Results: Three gel compositions were used. Sample 1 was prepared on gel basis including benzydamine in liquid form and demonstrated inhibitory effect towards pseudonormal murine BALB-3T3 fibroblasts and murine J774.2 macrophages, however, normal human HaCaT keratinocytes were resistant to its action. Sample 2 included BH in powder form and it did not affect significantly HaCaT keratinocytes and BALB-3T3 fibroblasts, but it suppressed J774.2 macrophages. Sample 3 («Benzidaflaziverdine») was developed and patented by us as a gel composed of benzydamine in powder form and flavonoid drops «Proteflazid®». It did not suppress tested mammalian cells and was not genotoxic (measured as % of DNA in comet tail and Olive Tail Moment) for murine J774.2 macrophages.

Conclusions: Inclusion of flavonoids in gel composition «Benzidaflaziverdine» blocked cytotoxic and genotoxic actions of benzydamine. Developed gel composition might be efficient in clinical periodontology, in particular, for treatment of periodontal diseases in orthodontic patients.

KEY WORDS: periodontium, gel composition, flavonoids, mammalian cell lines, biotolerance

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INTRODUCTION

The orthodontic anomalies and malocclusions rank third after caries and periodontal diseases (40 - 85% of cases), thus, this problem is both of medical and social significance [1-3]. The results of epidemiological studies show that the polyetiological nature of orthodontic anomalies and malocclusions and untimely diagnosis at the stage of formation in temporary and mixed occlusion encourages the development of their more severe forms in a permanent occlusion. Along with the orthodontic anomalies, the periodontal diseases is recognized as a multifactorial one that continue leading the list of causes of tooth loss in the adult patients [4].

Patients with the orthodontic anomalies of the periodontal pathology require standard hardware treatment lasting from 6 months to 2.5 years [5, 6]. In turn, the active period of the orthodontic treatment using braces can cause an recurrence of the periodontal disease during which a number of processes occur in the oral cavity associated with the lipid peroxidation (LPO), exposure to metals and long retention period which indicates the need for effective and targeted preventive and treatment measures, primarily before and during active phase of the orthodontic treatment [7-9].

Thus, a search for effective remedies of treatment and prevention of the periodontal diseases in the orthodontic patients continues. It is important that local medicines in the form of pastes, ointments, gel compositions should have an intense anti-inflammatory, immunomodulatory, anti-edematous, antiexudative, antimicrobial, fungicidal

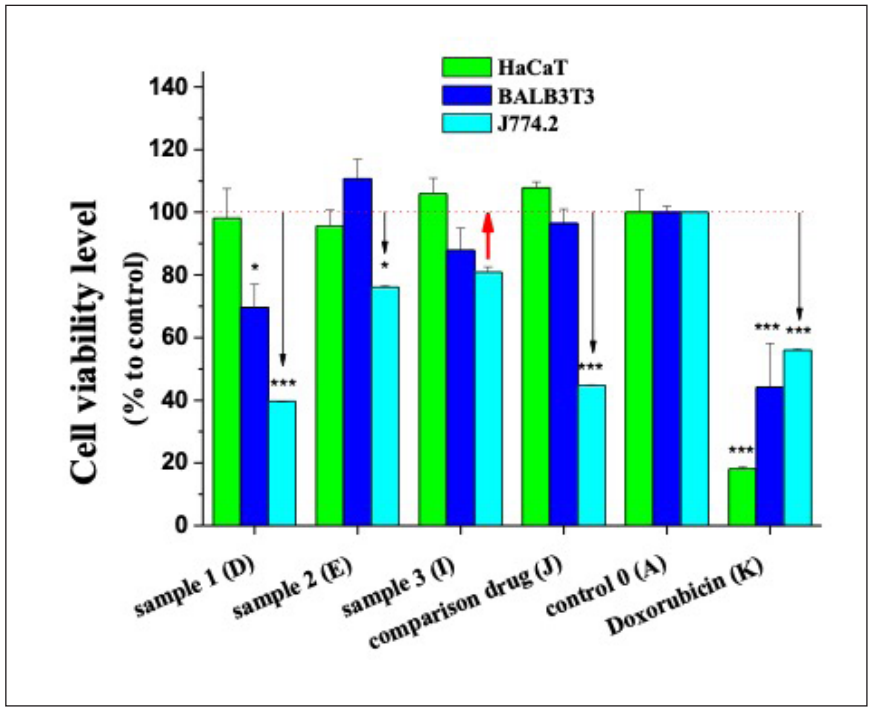

Fig. 1. Comparison of the viability rates (MTT-test, M ± m) of various lines of pseudonormal mammalian cells: murine fibroblasts of BALB-3T3 line, human keartinocytes of HaCaT line and murine macrophages of J774.2 line.
Notes: Control 0 – intact native cells, A (Table I); Comparison drug – «Cholisal» (Elfa, Poland), gel was added in the final concentration = 1%, J (Table I); Toxicological (positive) control – Doxorubicin, 1 μg/ml (Kyivmedpreparat, Ukraine), K (Table I)
Significance of difference with the non-treated control cells:
* P<0.05; *** P<0.001.

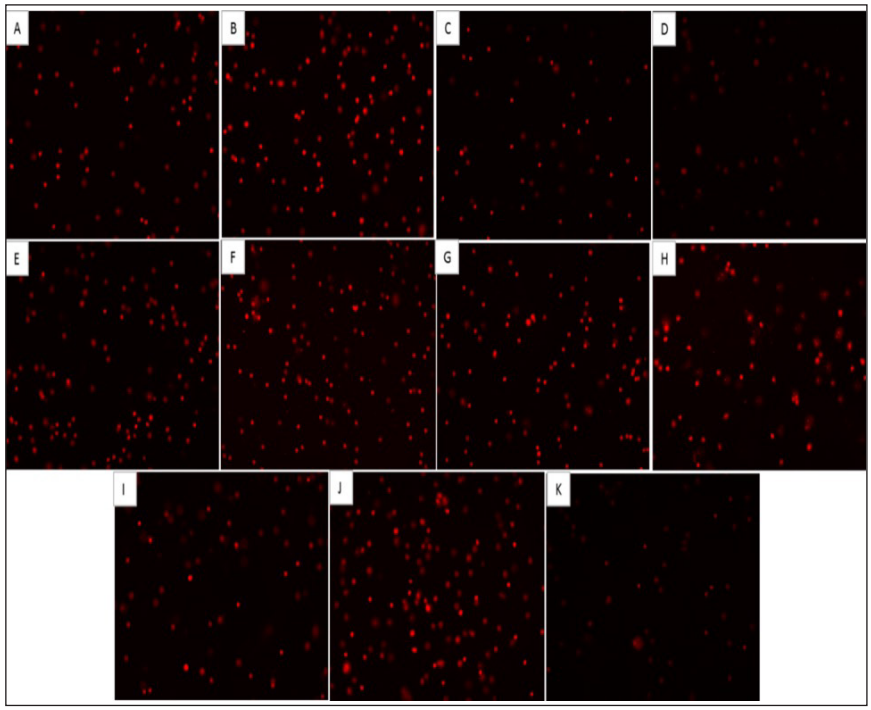

Fig. 2. The results of DNA comet study in murine J774.2 macrophages treated for 72 h with various gel compositions and other substances (A – control (native cell culture), B – DMSO (0,1%), C – gel base, D – Sample 1, E – Sample 2, F – gel base + BH (in solution and powder), G – gel base + «Proteflazid®», H – gel base + BH (in solution and powder) + «Proteflazid®», I – Sample 3 (patented gel composition), J – «Cholisal» (final concentration = 1%), K – Doxorubicin (1 μg/ml). Microscopic magnification x100.
Note: Final concentration of studied substances in culture medium with cells was 1%.

and antioxidant properties due to the improved composition and quantitative ratio of components that allow a reduction of treatment terms [10].

Flavonoids are known to possess a pronounced antioxidant effect. The phenolic structure of flavonoids allows them to interact with free radicals, reducing the intensity of lipid peroxidation (LPO) and inhibiting the malondialdehyde, that leads to a reduction of the level of local stress in tissues [11, 12].

In order to treat the periodontal diseases in the orthodontic patients, we have developed on the basis of the above drugs and patented the extemporaneous gel composition «Benzidaflaziverdine». This composition includes the BH (tablet form «T-Sept®»), «Proteflazid®» drops, as well as gel-based sodium alginate, nipagin and water for injections [13].

THE AIM

Different gels composed of benzydamine and flavonoids that were developed for treatment of the periodontal diseases in the orthodontic patients

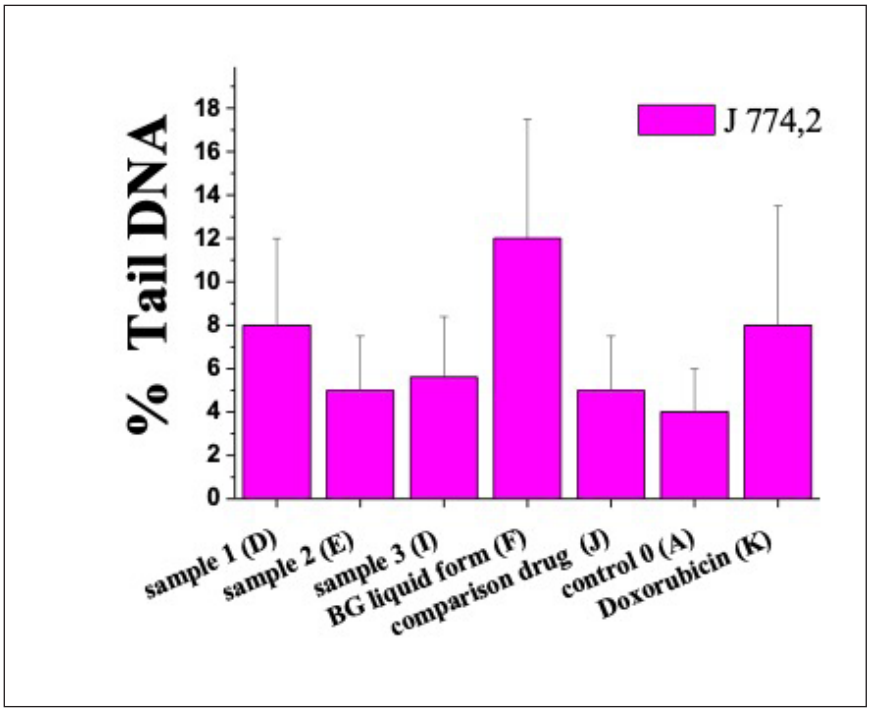

Fig. 3. The results of testing genotoxic effect of studied samples of various gel compositions introduced in the medium (0.5% dose in volumetric units) with cultured murine macrophages of J774.2 line (results are presented as a M ± m of 250 comets for each sample).
Quantitative evaluation of the results shown in Fig. 2.
Note: Control 0 – intact native cell culture, A (Table I); Comparison drug – «Cholisal», Elfa, Poland, (gel was added in the final concentration = 1%)), J (Table I); Toxicological (positive) control – Doxorubicin, 1 μg/ml (Kyivmedpreparat, Ukraine), K (Table I)

will be compared regarding their effects on survival (MTT-testing) of the mammalian cells of various tissue origin (fibroblasts, keartinocytes and macrophages).and the DNA intactness (DNA-comet assay) in treated cells.

MATERIALS AND METHODS

We compared the effect of different variants of gel composition «Benzidaflaziverdine» on the viability of mouse fibroblasts of the BALB-3T3 line, mouse macrophages of the J774.2 line, and human keratinocytes of the HaCaT line. Cell viability was assessed using MTT-reagent and genotoxic effects in treated cells were studied using DNA comet assay.

The components of the studied gel compositions which differed in the ratio and shape are presented in Table I. The applied samples included sodium alginate, nipagin and water for injections - gel base, BH in solution («Tantum Verde®») and its tablet form («T-Sept®»), drops «Proteflazid®». The untreated mammalian cells used as zero control were: murine fibroblasts of BALB-3T3 line, human keartinocytes of HaCaT line and murine macrophages of J774.2 line. Culturing of cells was performed in DMEM medium (Sigma Chem Co., USA) in the presence of decomplemented serum of cow embryos (Sigma Chem Co., USA) and 50 μg/ml gentamycin (Sigma, Chem Co., USA) in a thermostate with 5% CO_2 content at 37° C. Cells were sub-cultured every 2-3 days at 500 thousand to 1 million cells per 1 ml of culture medium [14, 15]. The number of viable cells was determined using a test with the MTT reagent according to the manufacturer's recommendations (Sigma, Chem Co., USA) [16, 17]. The principle of measuring the viability of cells is based on the ability of mitochondrial dehydrogenases of living cells to transform the unstained form the of the MTT reagent (3- (4,5-dimethylthiazol-2-yl) -2,5-dimethyl tetrazolium bromide, Sigma-Aldrich, USA) to crystalline blue formazan soluble in the dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA).

The main drug for comparison was a gel for topical treatment of the oral mucosa «Cholisal» (Jelfa S.A. Poland), whose active substances are: choline salicylate and cetalconium chloride. Doxorubicin (Kyivmedpreparat, Ukraine) was used as a prooxidant and Alfatocopherol (Technologist CJSC, Ukraine) – as an antioxidant.

To determine the cytotoxic effect of studied substances using the MTT-test, cells were seeded in 96-well plates (Greiner Bio One, Germany) at a concentration of $2x10^5$ / 100 μl in culture medium in the presence of 10% serum of cow embryos. After that, the studied substances were applied in different concentrations and kept for 72 h. 3 h before the end of the incubation time of the cells with the test substances in the medium was added 0.5% aqueous solution of MTT-reagent to a final concentration of 0.5 mg / ml. After incubation of the cells with the MTT reagent, the DMSO 100 μl was added to the wells in order to dissolve the formazan crystals formed during the reduction of the MTT-reagent by the viable cells. The concentration of formazan in wells was determined photometrically on a microphotometer «Plate Reader BioTek» 76883 (BioTek, USA) by measuring optical absorption at 490 nm. The ration of viable cells

Table I. Chemical components of gel compositions under study and preparations for comparison

Samples	Variants of the gel composition «Benzidaflaziverdine» and other tested substances
A	control (native cell culture)
B	DMSO (final concentration - 0,1%)
	Components of the gel composition (composition was added in the final concentration = 1%)
C	gel base – sodium alginate 5% (0.5 ml), nipagin (0.01 ml), water for injections (9.5 ml)
D	**Sample 1** – gel base (sodium alginate 5% (0.5 ml), nipagin (0.01 ml) water for injections (7.5 ml)), «Tantum Verde®» solution 0.15% (2 ml)
E	**Sample 2** – gel base (sodium alginate 5% (0.5 ml), nipagin (0.01 ml) water for injections (8.7 ml)), powder/tablet «T-Sept®» (0.73 g)
F	gel base (sodium alginate 5% (0.5 ml), nipagin (0.01 ml), water for injections (8.1 ml)), «Tantum Verde®» solution 0.15% (1 ml), powder/tablet «T-Sept®» (0.365 g)
G	gel base (sodium alginate 5% (0.5 ml), nipagin (0.01 ml), water for injections (8 ml)), «Proteflazid®» (1.5 ml)
H	gel base (sodium alginate 5% (0.5 ml), nipagin (0.01 ml), water for injections (6.6 ml)), «Tantum Verde®» solution 0.15% (1 ml), powder/tablet «T- Sept®» (0.365 g), «Proteflazid®» (1.5 ml)
I	**Sample 3** (patented gel composition «Benzidaflaziverdine») – gel base (sodium alginate 5% (0.5 ml), nipagin (0.01 ml, water for injections (8 ml)), powder/tablet «T-Sept®» 0.73 g), «Proteflazid®» (1.5 ml)
J	Cholisal (gel was added in the final concentration = 1%)
K	Doxorubicin (final concentration – 1 μg/ml)

Table II. DNA damage effect in murine J774.2 macrophages under their treatment (72 h) with various substances under study (% Tail DNA and Olive Tail Moment)

	Variants of gel composition «Benzidaflaziverdine» and other substances										
Substances (see: Fig. 2)	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Parameters, percentage of DNA in the Tail	4%	8%	6%	8%	5%	7%	3%	12%	6%	5%	8%
Olive Tail Moment	0.33	0.33	0.69	1.3	0.4	0.61	0.25	0.48	0.4	0.56	0.57

was expressed as a percentage relative to the control (untreated cells), which was taken as 100% [14, 15].

GENOTOXICITY TESTING

The method of DNA-comets under alkaline conditions was used [18, 19]. Cells (1 million / ml) were incubated for 24 h with test samples. The cell suspension (3×10^4) was mixed at 37 ° C with 250 μl of 0.5% fusible agarose solution (Sigma, USA) and applied in a thin layer on slides, which were pre-coated with 1% agarose solution (Sigma, USA) and dried. Cell lysis and electrophoresis were performed, as described [19]. DNA comets were stained with the Ethidium bromide (10 μg / ml) and examined under a Carl Zeiss fluorescence microscope (Germany). Assessment of DNA damage was performed using the program «CASP» 1.2.2 (CASPlab, Wroclaw, Poland), comparing the average values (%) of DNA in the tail of 250 comets for each sample [18, 19]. Calculation of the relative units of the Olive Tail Moment (OTM) for quantification of the induced DNA fragmentation (damage) was performed as: OTM = (Tail.mean - Head. mean) × (Tail % DNA)/100.

Bioethics was kept in accordance with requirements of the basic bioethical provisions of the European Convention on Human Rights and Biomedicine (from 04.04.1997) and Helsinki Declaration of the World Medical Association on the ethical principles for medical research involving human subjects (1964-2008). Protocol N9 dated by 21.12.2020 and Protocol N8 dated by 18.10.2021were approved by the Committee on BioEthics of scientific research, experimental development and scientific works at Danylo Halytskyi Lviv National Medical University.

STATISTICAL PROCESSING OF RESULTS

The experiments were performed in three parallels in each variant. Each indicator shown in the figures (the ordinate of the columns in the diagrams) corresponds to the average value of «M» calculated from the results of three measurements in one of several experiments of the same type. The error «m» of the obtained result was calculated by the value of the root square error «σ». This is represented on the illustration near each indicator by a vertical line, the length of which corresponds

to the value of «m». The computer analysis was based on Phenom II X4 975 with Windows 7 64-bit operating system (Microsoft, USA). Statistical processing of the obtained data was performed in MS Excel 2010 (Microsoft, USA). The P-value of ≤0.05 was considered as statistically significant.

RESULTS

Various modifications of gel composition «Benzidaflaziverdine» and drugs for comparison (Samples A-K: see Table I) were studied. The application of methods for assessing viability in cell populations and estimating genotoxic effects (DNA comet assay) showed that Substance C was toxic to all used cell lines. The level of its cytopathic effect is comparable to the effect of antitumor drug Doxorubicin (Substance K) at a concentration of 1 μg per 1 ml of culture medium. These results are presented in Figures 1 and 2.

Substance D was found to inhibit the viability of BALB-3T3 fibroblasts and even stronger inhibition was observed for J774.2 macrophages, while HaCaT keratinocytes were ersistant to the action of this substance. Substances E, F, G, H and J («Cholisal» – the drug for comparison) did not have a statistically significant effect on keratinocytes and fibroblasts, but inhibited the macrophage of J774.2 line. It should be noted that the inhibitory effect of «Cholisal» (Substance J) towards J774.2 macrophages was more pronounced than such effect of highly toxic anticancer drug Doxorubicin (Substance K) that was used at 1 μg per ml dose. An additional study is necessary to answer a question whether this action «Cholisal» was cytotoxic, cytostatic, or functionally suppressive.

On Figure 1, a quantitative assessment of the impact of studied substances on the viability of various pseudonormal mammalian cells *in vitro* is demonstrated. As one can see, the action of Sample 3 (I) which is a patented gel composition «Benzidaflaziverdine» containing gel-base + «Proteflazid®» + «T-Sept®», was the least inhibitory for the macrophages, with acceptable characteristics of its effect on the keratinocytes and fibroblasts. It might be suggested that this Sample 3 (I) possessed the highest regenerative potential for tissue cells as compared to Sample 2 (E), Sample 1 (D) and the «Cholisal» (J) – drug for comparison. Further studies are required to explain why the «Cholisal» is so toxic for the mammalian macrophages.

According to the results of the MTT-testing, it was found that sample D (Sample 1), which included gel base and BH in liquid form («Tantum Verde®»), was toxic for all cell lines, inhibited the viability of fibroblasts of the BALB-3T3 line and even more – the macrophages J774.2, but did not influence significantly on the keratinocytes of HaCaT line. The sample E (Sample 2), which contains the BH in the form of powder («T-Sept®»), did not have a significant effect on the keratinocytes and fibroblasts, but suppressed the viability of the macrophages of J774.2 line. It should be noted that the comparison drug «Cholisal» (J) was even stronger inhibitor of macrophages of J774.2 line than the Doxorubicin used at 1 μg per ml dose.

Since the macrophages of J774.2 line were found to be most sensitive to cytotoxic effect of the proposed samples of various gel compositions, these mammalian cells were selected for estimation of the DNA damaging effect of those compositions. In general, the most cytotoxic samples were also found to be the most genotoxic ones. The patented by us gel composition I (Sample 3) that did not demonstrate a pronounced cytotoxic effect, was also lacking the genotoxic effect in the macrophages of J774.2 line (Figure 2, Table II). This effect was measured as a percentage of DNA in the comet tails and as a value of the Olive Tail Moment (OTM).

Different variants of gel compositions, namely sample C (gel base), Sample 2 (E – gel base + «T-Sept®»), sample G (gel base + «Proteflazid®»), Sample 3 (I – («Benzidaflaziverdine» – gel-base + «Proteflazid®» + «T-Sept®») and the comparison drug «Cholisal» (J) were compared regarding their ability to cause the DNA damage in murine macrophages of J774.2 line. We did not reveal any significant genotoxic effect in these cells. The indicators of DNA damage estimated as a percentage of DNA in the tail were 6%, 5%, 3%, 6%, 5%, respectively, and 4% in control, and the values of the Olive Tail Moment (OTM) were 0.69, 0.4, 0.25, 0.4, 0.56 relative units, respectively, and 0.33 relative units in control.

The maximum amount of DNA in the comet tail (12%) was observed in cells treated with the gel composition H, which contained BH both in powder form and in liquid form in a residual amount. At the same time, the patented gel composition I («Benzidaflaziverdine») (Sample 3) caused significantly (2 times) less DNA damage (Table II). Thus, the proposed gel composition I (Sample 3), as well as the gel composition G («Proteflazid®» in combination with a gel base) demonstrated the DNA protecting action (Table II, Figures 2 and 3).

In Figure 3, quantitative evaluation of the results presented in Figure 2 is shown.

As can be seen from the results presented in Figure 3, the studied gel compositions were not genotoxic (% of DNA in Comet Tail and Olive Tail Moment) for murine macrophages of J774.2 line. Those biotolerance indicators (level of cell viability suppression and genotoxic effect) were comparable with the effect of «Cholisal» used in the experiments as a drug for comparison.

DISCUSSION

Application of the orthodontic treatment in patients with predisposition to periodontal diseases is an important step, because the use of fixed orthodontic techniques may cause stress-induced metabolic disorders in the periodontal tissues due to the links between the oxidative stress and inflammatory response. As a result, redox status may provoke a progressive loss of the periodontal tissues, in particular, bone tissue of the alveolar process [20-24].

That is why the development of novel complex medicines for treatment of patients with periodontal diseases that in addition to dental tissue protecting agents also include the biologically active agents of 2nd line of defence (supportive treatment) is of great significance in dentistry and periodontology.

The «Proteflazid®» (LLC NKV «ECOPHARM», Ukraine) is recommended for treatment of viral, bacterial and fungi infections. According to the manufacturer's instructions, it is based on flavonoids and possesses immunotropic properties, protects mucous membranes, and normalizes local immunity (levels of lactoferrin, secretory immunoglobulin A, lysozyme and C3 component of complement). This drug is used in the form of drops. It is an inducer of synthesis of endogenous α- and γ-interferons to physiologically active levels. It increases the non-specific resistance of the body to viral and bacterial infections, has antioxidant activity, does not demonstrate immunotoxic effects and does not cause the refractoriness (hyporeactivity) of the immune system.

Along with flavonoids, a number of the non-steroidal anti-inflammatory drugs (NSAIDs) is recommended for topical use in dentistry and periodontology are available on the pharmaceutical market. Although they are less effective than steroids, but they are not as toxic as the steroid hormones. In our study, we addressed our attention to the benzydamine hydrochloride (BH) that is a low-toxic non-steroidal drug with high lipophilicity. It penetrates well into the sites of inflammation, where the pH is lowered, and, accordingly, creates a therapeutic concentration there, accelerating the reparative processes. The severity of the local analgetic effect of the benzydamine provided by a direct membrane-stabilizing effect on sensitive nerve endings, exceeds similar action of most NSAIDs. In addition, BH demonstrates the ability to inhibit the adhesion of leukocytes to vascular endothelium, blocks platelet adhesion factor, improves capillary permeability, which ensures its vasoprotective effect [25]. The antibacterial action of benzydamine is caused by its rapid penetration through the membranes of microorganisms, damage of their cellular structures, disruption of the metabolic processes and cell lysis. The fungicidal action of the BH is realized via structural modifications of fungi cell wall and metabolic chains of the mycelium, thus, preventing their reproduction, against 20 strains of fungi of *Candida albicans* and *Candida tropicalis*, as well as *Aspergillus niger*. The representatives of the BH-based drugs are «T-Sept®» lozenges (ICN, Polfa, Poland) and «Tantum Verde®» mouthwash (Angelini Francesco A.C.R.A.F, S.p.A., Italy) [26, 27].

Here, we conducted a comparative study of cyto- and genotoxicity of different variants of gel compositions proposed for the treatment of the periodontal diseases in the orthodontic patients. The results of our study demonstrated that the composition G («Proteflazid®» in combination with gel base) and composition I (Sample 3 – «Benzidaflaziverdine») were the least toxic to the mammalian cells of various tissue origin. Besides, these compositions were found to possess the highest DNA protective activity that was evidenced by the lowest degree of DNA damage (normal shape of nuclei with minimal damage) compared to control (untreated cells).

Other applied gel compositions were less biocompatible and the comparison drug «Cholisal» was even more suppressive for the mammalian macrophages than composition G and composition I. Additional study is necessary to explain whether the action of «Cholisal» (comparison drug, J) is cytotoxic, cytostatic, or inhibits the functional activity of cells.

Composition I (Sample 3 – «Benzidaflaziverdine») composed of the flavonoids and non-steroidal anti-inflammatory agents of non-steroidal nature was sufficiently biotolerant. In other investigations [28, 29] using experimental models of rodents, a diverse effect of flavonoids on periodontal cells and tissues was found, including regulation of the inflammatory response and potential conservative effects in periodontal ligaments and bone tissue of the jaws.

It can be assumed that the effect of flavonoids is beneficial in combination with the nonsteroidal anti-inflammatory agent benzydamine hydrochloride on various types of periodontal cells, including gum epithelial cells, gum fibroblasts and periodontal ligament fibroblasts, and on osteoblasts. Therefore, the flavonoids in a combination with benzydamine hydrochloride in powder form are a promising clinical tool in the prevention and treatment of gingivitis and periodontitis. Gel composition proposed in our study might be recommended for use in clinical periodontology, in particular for treatment of periodontal pathology in orthodontic patients, both in preparation for orthodontic treatment and in its active period.

CONCLUSIONS

It was found that the presence of flavonoids in the gel composition «Benzidaflaziverdine» minimizes the cytotoxic

and genotoxic action of benzydamine hydrochloride, which allows to more fully realize its antimicrobial and anti-inflammatory action. We do not recommend the benzydamine hydrochloride in liquid form, in contrast to the tablet drug, for inclusion in prolonged forms of gel compositions used for the local treatment of gingivitis and periodontitis.

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ORIGINAL ARTICLE

ASSESSMENT OF THE RISK OF ADVERSE EFFECTS OF DIFFERENT PESTICIDE GROUPS FOR HUMANS CONSUMING APPLES AND GRAPES TREATED WITH PESTICIDES

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ABSTRACT

The aim: To assess the risk of adverse effects of various groups of pesticides for humans, consuming apples and grapes (treated by pesticides).

Materials and methods: The gas-liquid chromatography, high-performance liquid chromatography, atomic absorption spectroscopy and tandem chromatography–mass spectrometry methods were used for the quantitative calculation of pesticides in apples and grapes. The possible intake of pesticides (with mentioned products) and the integral indicator of danger during their consumption were considered, while assessing the risk for the people consuming apples.

Results: It has been proven, that the processes of pesticide decomposition in growing agricultural crops (apples, grapes) occur according to an exponential model. The half-life periods of the studied pesticides in agricultural plants were established. And in terms of stability, the studied substances are moderately stable. An exception is bifenthrin – a persistent pesticide. The calculated risk values of dangerous exposure to pesticides, when consuming apples and grapes, treated with pesticides, were 2-3 orders of magnitude lower than the permitted level, and ranged from 2.0×10^{-3} to 7.8×10^{-2}. Most pesticides are moderately dangerous, according to the value of the integrated indicator of danger, during the product consumption, except for the kresoxim-methyl and clothianidin, which are not very dangerous.

Conclusions: The obtained results should be taken into account, considering the issue of expanding the pesticides application field, based on the studied substances, and the necessity for monitoring studies.

KEY WORDS: Consuming apples and grapes, The following pesticides, The obtained results

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INTRODUCTION

Apples and grapes are the main sources of vitamins and trace elements, compared to other fruit in Ukraine. This means, that the consumption of these products and their juices gives us the opportunity to obtain unique phytobioactive substances (polyphenols, anthocyanins, lycopene, resveratrol, beta-carotene, quercetin, naringin, nobiletin, caffeic acid, gallic acid, etc.). This group of products provides benefits for physical and mental health, and also takes part in the prevention of various non-communicable diseases (cardiovascular, neurological diseases, obesity, diabetes, osteoarthritis, and some types of cancer) [1, 2].

However, regardless of the benefits we get from eating apples and grapes, there is a possibility of dangerous exposure to pesticides, applied in the cultivation of agricultural crops, including fruit. It is known that pesticides can cause acute and chronic poisoning, being a risk factor for the development of oncological pathology, nervous and reproductive system diseases, as well as endocrine disorders [3, 4].

As of 2022, about 2,450 pesticide formulations have been registered on the territory of Ukraine, 13.4% of these substances are recommended for growing apples and 10.7% - grapes [5]. The number of pesticide formulations proposed for use is constantly increasing, which increases the risk for the population. Therefore, it is relevant to predict the danger, which comes with the consumption of agricultural products, when new pesticides are used.

THE AIM

The purpose of the study is to assess the risk of adverse effects of various groups of pesticides for humans, consuming apples and grapes (treated with pesticides).

MATERIALS AND METHODS

Field studies have been conducted for 10 years on the basis of the Institute of Hygiene and Ecology, valuing the dynamics of the content of different groups of

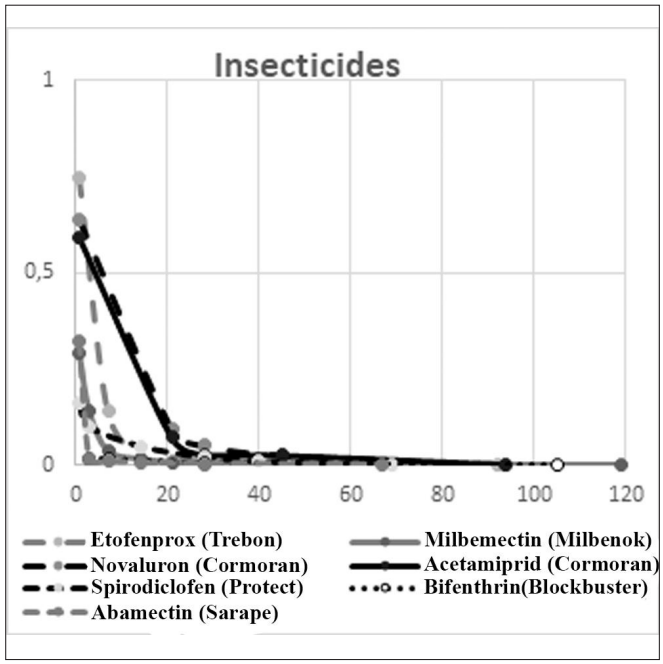

Fig. 1. Dynamics of the content of studied insecticides in apples

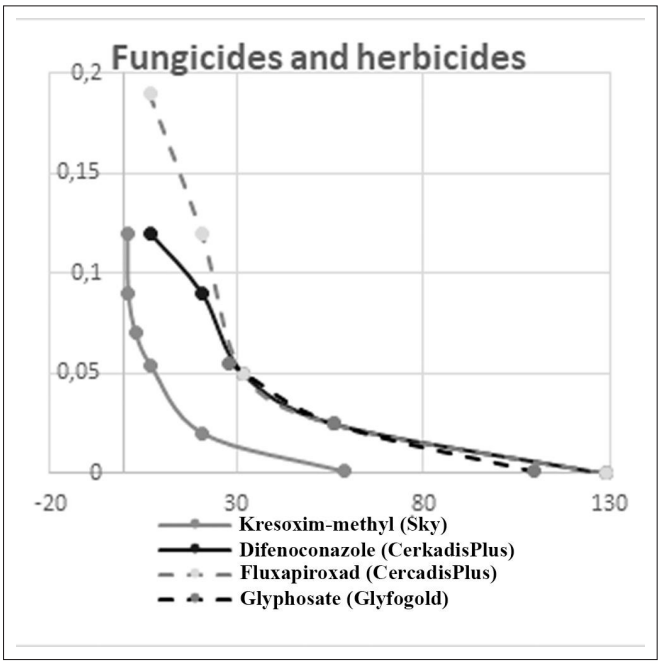

Fig. 2. Dynamics of the content of studied fungicides and herbicides in apples

pesticides in apples and grapes, in different soil and climate regions of Ukraine. The following pesticides formulations, used on apple trees and vineyards, were studied– insecticides Milbanok (active substance (a.s.) milbemectin), Trebon (drug etofenprox), Kormoran (drug acetamiprid, novaluron), Protect (drug spirodiclofen), Blockbuster (a.s. bifenthrin), Sarape (a.s. abamectin), TurboPresto (a.s. clothianidin, lambda-cyhalothrin), fungicides: Lifesul (a.s. sulfur), Bluestar (a.s. copper), Sky (a.s. kresoxim-methyl), Cerkadis Plus (a.s. difenoconazole, fluxapiroxad), herbicides: Zumer (a.s. glyphosate, oxyfluorfen), Glyfogold (a.s. glyphosate) in the industrial sector. The research was performed in the Institute of Hygiene and Ecology of the National Medical University named after O.O. Bogomolets, under accreditation DSTU ISO/IE 17025:2017.

The gas-liquid chromatography, high-performance liquid chromatography, atomic absorption spectroscopy and tandem chromatography–mass spectrometry methods were used for the quantitative determination of pesticides (Table I).

Mathematical modeling of the rate of destruction (the period of decomposition of the substance by 50% (T50), by 95% (T95), the constant of the rate of destruction (K), days) of the studied pesticides was performed using the exponential model, according to the first-order equation, and the calculation of the coefficient of determination (R^2).

Predicting the danger to the population consuming apples and grapes, treated by the chemical protection agents, was performed according to the methods proposed by specialists of the Institute of Hygiene and Ecology [6, 7].

The results were treated statistically, using the package of statistical programs IBM SPSS StatisticsBase v.22 and MS Excel. Descriptive statistics was used in the statistical analysis of the obtained data; comparison of average values of variables was carried out using parametric and non-parametric methods. Differences with a significance level of more than 95% ($p<0.05$) were considered reliable.

RESULTS

The first stage of our research was to determine the organoleptic properties of products, treated by the studied drugs.

Control samples were taken from areas, where untreated apple trees and vineyards were grown.

It was established that the organoleptic properties of the harvested apples and grapes (smell, color, appearance) did not differ from the control samples. The content of the studied active substances in apples and grapes was determined simultaneously with the assessment of organoleptic properties.

The analysis of the obtained results, regarding the content of the residues of the analyzed pesticides in the samples of apples and grapes, showed a gradual decrease in the content of the investigated fungicides and insecticides in the leaves and fruits.

Regarding application of the insecticides Milbenok, Trebon, Kormoran, Protect, Blockbuster, Sarape in the period after the blooming period, it was established that the initial concentrations of milbemectin in leaves were 0.29 mg/kg, apples - 0.037-0.042 mg/kg, etofenprox in

apples - 0 ,24-0.14 mg/kg, acetamiprid and novaluron in leaves 0.59 mg/kg and 0.64 mg/kg, respectively, in apples 0.071 mg/kg and 0.094 mg/kg, respectively, spirodiclofen in apples - 0 .16 mg/kg, bifenthrin - 0.017 mg/kg, abamectin in leaves - 0.32 mg/kg, apples - 0.016 mg/kg. In the consequent periods it decreased, and during harvesting, did not exceed the limit of quantitative determination, according to the corresponding analytical method.

Field studies of the fungicides Lifesul, BlueStar, Cerkadis Plus, and Sky showed that the initial concentrations of sulfur in the fruit were 35.5 mg/kg, copper – 1.9 mg/kg, difenoconazole – 0.12 mg/kg, fluxapiroxad – 0, 19 mg/kg, kresoxim-methyl – 0.12 mg/kg. Until the 28th day of the study, the residual amounts of the studied fungicides did not exceed the limit of quantitative determination of the analytical method (Figs 1 and 2).

The studied herbicides were not detected in the fruits during all the periods of the study, which is explained by the method of application of Glifogold and Zoomer - application between the rows of apple orchards.

Field studies of Milbank and Protect insecticides were conducted in vineyards. The initial concentrations of the active substances of the studied insecticides were 0.044-0.051 mg/kg for milbemectin in grape berries, 0.45 mg/kg for leaves, and 0.25 mg/kg for spirodiclofen in berries. The analyzed active substances were determined in an amount below the limit of quantitative determination of the analytical method after 28-40 days of observation.

The initial concentrations of the active substances of the studied fungicides BlueStar and Sky for copper were 2.7 mg/kg, kresoxim-methyl - 0.19 mg/kg in berries, and 1.33 mg/kg kresoxim-methyl in grape leaves. In the subsequent periods of observation, the remaining amounts of the studied compounds were below the limits of quantitative determination of the corresponding method. Residues of the active substances glyphosate and oxyfluorfen herbicides Glyfogold , Zumer were not detected in grapes during all periods of the study, which is explained by the method of plant treatment (treatment between rows of vineyards).

Mathematical analysis of the obtained results of the studied active substances content dynamical pattern, in apples, during treatment by pesticides, made it possible

Table I. Studied pesticides' terms of use general characteristics and analysis methods

Pesticide	Consumption rate, l/ha (kg / t), (multiplicity)	Culture	Application technology	Active substance	MGD [Approval No.]	LQD /LD, mg/kg	
						apples	grapes
Zoomer	3.0 (1)	apple tree, grapes	Application between the rows of apple orchards/ vineyards	oxyfluorfen	GLC [3063-84]	0.04 / 0.01	0.04 / 0.01
				glyphosate	HPLC [363-2002]	0.1 /0.03	0.05 /0.02
Glyfogold	8.0 (1)			glyphosate	HPLC [363-2002]	0.1 /0.03	0.05 /0.02
Milbenok	1.0 (3)	apple tree, grapes	Applied during the growing season	milbemectin	HPLC [1763-2021]	0.02/0.008	0.02/0.008
Sky	0.2 (3) 0.3 (3)			kresoxim-methyl	GLC [205-2000]	0.05/0.02	0.05/0.02
Protect	0.6 (2) 0.4 (2)			spirodiclofen	HPLC [1024-2010]	0.02/0.008	0.02/0.008
Blue star	2.0 (4) 3.0, (4)			copper	AAS [24-97, 527-2004]	1.0/0.3	1.0/0.3
Trebon	0.5 (3)	apple		etofenprox	TXMC [1799-2022]	0.01/0.003	-
Cormorant	0.8 (2)			acetamiprid	HPLC [197-2000]	0.025/0.008	-
				novaluron	HPLC [302-2001]	0.05/0.02	-
Laifsul	6.0 (2)			sulfur	HPLC [292-2001]	5.0/2.0	-
Blockbuster	0.5 (2)			bifenthrin	GLC [6207-91]	0.05/0.02	-
Serkadis Plus	1.5 (3)			difenoconazole	GLC [55-97]	0.05/0.02	-
				fluxapiroxad	HPLC [1514-2018]	0.05/0.02	-
Sarape	1.5 (3)			abamectin	HPLC [1109-2011]	0.005/0.002	-
Harvest Smart	0.00035 (1)	apple	Applied when stored	1-methyl-cyclopropene	GLC [794-2007]	0.01/0.003	-

Notes: 1. MGD – methodical guidelines for the determination of pesticides; 2. LQD – the limit of quantitative determination; 3. LD – limit of detection; 4. HPLC – high performance liquid chromatography; 5. GLC - gas-liquid chromatography; 6. AAS – atomic absorption spectroscopy; 7. TCMS - tandem chromatography -mass spectrometry.

Table II. Degradation indicators of studied pesticides in apples (in the agro-industrial sector) (n=12)

Active substance (Pesticide)	Kinetic equation	K (day $^{-1}$)	T50 (day)	T95 (day)	R^2
Insecticides					
Milbemectin (Milbenok)	$y = 0.0677e^{-0.059x}$ $y = 0.0714e^{-0.074x}$	0.059 ±0.002	11.8 ± 0.6	51.3±2.5	0.86 0.93
Etofenprox (Trebon)	$y = 0.1299e^{-0.067x}$ $y = 0.0665e^{-0.106x}$	0.086±0.009	8.4±0.9	36.7±3.7	0.78 0.78
Acetamiprid (Cormoran)	$y = 0.3297e^{-0.062x}$	0.062±0.001	11.2±0.2	48.7±0.9	0.94
Novaluron (Cormoran)	$y = 0.4451e^{-0.064x}$	0.064±0.001	10.7±0.2	46.6±0.7	0.98
Spirodiclofen (Protect)	$y = 0.163e^{-0.08x}$	0.079±0.001	8.6±0.1	37.5±0.4	0.98
Bifenthrin (Blockbuster)	$y = 0.0196e^{-0.038x}$	0.038±0.001	18.1±0.1	78.5±0.7	0.99
Abamectin (Sarape)	$y = 0.0401e^{-0.092x}$	0.057±0.001	12.1±0.2	52.5±1.0	0.80
Fungicides					
Sulfur (Lifesul)	$y = 33.871e^{-0.003x}$	0.028±0.001	24.6±1.0	106.9±4.4	0.70
Copper (Bluestar)	$y = 2.0068e^{-0.047x}$	0.047±0.003	14.7±0.9	64.2±3.8	0.95
Kresoxim-methyl (Sky)	$y = 0.1146e^{-0.076x}$	0.076±0.001	9.0±0.2	138.6±9.1	0.95
Difenoconazole (CerkadisPlus)	$y = 0.3026e^{-0.058x}$	0.058±0.001	11.9±0.1	51.6±0.6	0.97
Fluxapiroxad (CercadisPlus)	$y = 0.406e^{-0.061x}$	0.061±0.001	11.3±0.1	49.1±0.3	0.98
Herbicides					
Glyphosate (Zoomer)	$y = 1474.2e^{-0.165x}$	0.161±0.002	4.3±0.06	18.6±0.3	0.95
Oxyfluorfen (Zoomer)	$y = 650.96e^{-0.154x}$	0.248±0.002	2.8±0.02	12.1±0.09	0.89
Glyphosate (Glyfogold)	$y = 0.2623e^{-0.048x}$	0.077±0.001	8.9±0.1	38.9±0.3	0.98

Notes: 1. K - pesticide degradation rate constants; 2. T50 - half-life of pesticide; 3. T95 - the period of destruction of the pesticide by 95%; 4. R2 – coefficient of determination.

to establish the constants of the rate of degradation (K, days), the half-life period (T50), and the destruction of 95% (T95) of the active substances in apples (Table II).

Degradation indicators of the studied pesticides in apples, grown in orchards in the industrial sector, showed that the value of the coefficient of determination (R^2) was ranged within 0.70 - 0.99, which indicates a reliable relationship between the selected variables, and the selected exponential model is chosen , when modeling the results of the dynamical pattern of the studied pesticides' content.

Mathematical modeling of the obtained results of the studied pesticides' content dynamical pattern showed that the half-life (T50) of most of the analyzed insecticides was 8.4-12.1 days, organic fungicides 9.0-11.9 days, herbicides 2.8-8.9 days, which indicates their moderate persistence in grown agricultural crops (3rd class of danger, according to Sanitary Rules and Standards (DsanPiN) 8.8.1.002-98).

The insecticide bifenthrin and inorganic fungicides (copper and sulfur) had a T50 of 14.7-24.6 days, which made it possible to classify the analyzed compounds as persistent (3rd class of danger).

Similar studies were conducted, when pesticides were used in vineyards. The concentrations of the analyzed groups of pesticides in the initial period of the study were for milbemectin 0.45 mg/kg, spirodiclofen – 0.25 mg/kg, copper – 2.7 mg/kg, kresoxim-methyl – 1.33 mg/kg, glyphosate – < 0.05 mg/kg, oxyfluorfen - <0.04 mg/kg, when carrying out natural experiments in the agro-industrial sector (Table II).

It was found, that during growing of the vineyards, the concentrations of the studied groups of pesticides decreased. The process of pesticide decomposition in grapes, according to an exponential model (determination coefficient (R^2) = 0.92-0.99) (Table III), which is similar to the dynamical pattern of pesticides decomposition in apples.

Table III. Indicators of degradation of studied pesticides in grapes (n=12) (in the agro-industrial sector and personal subsidiary farms)

Active substance (drug)	Kinetic equation	K (day $^{-1}$)	T50 (day)	T95 (era)	R ^{2}
Insecticides					
Milbemectin (Milbenok)	$y = 0.1441e^{-0.071x}$ $y = 0.0774e^{-0.065x}$	0.068 ±0.001	10.3 ± 0.6	44.6±1.0	0.92 0.98
Spirodiclofen (Protect)	$y = 0.2766e^{-0.084x}$	0.084±0.001	8.2±0.1	35.6±0.4	0.95
Fungicides					
Copper (Bluestar)	$y = 2.8401e^{-0.035x}$	0.035±0.003	20.1±0.8	87.4±3.7	0.99
Kresoxim-methyl (Blue Sky)	$y = 0.8788e^{-0.098x}$	0.098±0.002	7.0±0.1	30.6±0.5	0.98
Herbicides					
Glyphosate (Zoomer)	$y = 11.651e^{-0.075x}$	0.085±0.001	8.4±0.9	36.3±3.9	0.95
Oxyfluorfen (Zoomer)	$y = 6.5372e^{-0.074x}$	0.082±0.008	8.5±0.8	37.1±3.3	0.97
Glyphosate (Glyphogold)	$y = 20.981e^{-0.084x}$	0.077±0.008	9.3±1.2	40.4±4.9	0.99

Notes: 1. K - pesticide degradation rate constants; 2. T50 - half-life of pesticide; 3. T95 - the period of pesticide destruction by 95%; 4. R2 – coefficient of determination.

Table IV. Risk assessment of pesticides adverse effects on human health when consuming apples and grapes treated by them

Active substance	PDD, mg/kg	T_{50} in plants, day [1]	T_{50} in plants, day [2]	average consumption of fruits, berries, g/day	IIPS		R
					value, points [1/2]	class [1/2]	
milbemectin	0.003	11.0 ±0.4* //	5.1 ±0.1		3+2 /2 +2=7 /7	3/3 _	2.6×10 $^{-2}$
etofenprox	0.003	8.4 ±0.9* //	2.1 ±0.1		3+2 /1 +2=7 /6	3/3 _	2.6×10 $^{-2}$
acetamiprid	0.010	11.2 ±0.2 //	7.5 ±2.3		2+2 /2 +2=6 /6	3/3 _	2.0×10 $^{-2}$
novaluron	0.010	10.7 ±0.2 //	6.7 ±2.8		2+2 /2 +2=6 /6	3/3 _	3.9×10 $^{-2}$
1-methylcycloprene	0.0009	-	-		-	-	4.3×10 $^{-2}$
spirodiclofen	0.001	8.4 ±0.1	10.0 ±0.1		4+2 /2 +2=8 /8	3/3 _	7.8×10 $^{-2}$
bifenthrin	0.020	18.1 ±0.1 //	8.9 ±4.1		2+3 /2 +2=7 /6	3/3 _	3.9×10 $^{-2}$
difenoconazole	0.002	11.7 ±0.1	12.2 ±6.8	164	3+2 /2 +2=7 /7	3/3 _	2.0×10 $^{-1}$
fluxapiroxad	0.020	11.3 ±0.1	10.0 ±0.6		2+2 /2 +2=6 /6	3/3 _	9.8×10 $^{-3}$
abamectin	0.0002	12.1 ±0.2	9.5 ±4.7		4+2 /2 +2=8 /8	3/3 _	3.9×10 $^{-1}$
glyphosate	0.010	7.7 ±0.7	9.9 ±4.3		2+2 /2 +2=6 /6	3/3 _	1.2×10 $^{-1}$
kresoxim-methyl	0.100	8.0 ±0.5	7.1 ±1.6		1+2 /2 +2=5 /5	4/4 _	2.0×10 $^{-3}$
oxyfluorfen	0.003	5.7 ±1.3	3.1 ±1.1		3+2 /1 +2=7 /6	3/3 _	1.3×10 $^{-1}$
clothianidin	0.080	7.9 ±0.2 //	14.3 ±8.0		1+2 /2 +2=5 /5	4/4 _	2.4×10 $^{-3}$
lambda-cyhalothrin	0.003	7.6 ±0.1 //	19.9 ±14.8		3+2 /3 + 2 =7 /8	3/3 _	1.3 × 10 $^{-2}$

Notes: PDD - permissible daily dose, mg/kg; IIPS – an integrated index of product safety; 1 – according to research data in the soil and climatic conditions of Ukraine; 2 according to the data of studies in countries of the EU [9]; * - differences are reliable between the persistence of pesticides in the soil and climatic conditions of Ukraine and EU countries according to the Student's criterion at $p \leq 0.05$; // - differences are reliable according to the z -criterion at $p \leq 0.05$; R - risk values.

The analysis of the danger of the investigated pesticides groups, according to the indicator of persistence in grown agricultural crops (grapes), showed that the danger period duration, using pesticides based on copper T50, in the the agro-industrial sector, is 20.1 days, in private households - 16.6 days. According to this criterion, the substance belongs to persistent compounds (2nd class of danger). T50 of milbemectin, spirodiclofen, kresoxim-methyl fungicide, glyphosate and oxyfluorfen herbicides was within the range of 7.0-10.3 days, which stands for moderate resistance in grapes (class 3, according to DSanPiN 8.8.1.002-98) [8].

DISCUSSION

The described methods were used to assess the risk to the population, consuming apples and grapes, treated by the investigated pesticides [6, 7].

At the first stage, the permissible daily intake with food products, and the possible intake of pesticides with the analyzed products, were calculated (Table IV). The calculation results showed that the risk values were in the range from 2.0×10^{-3} to 7.8×10^{-2}, which is much lower than the permissible value.

At the next stage, assessment of the danger to the people, consuming apples and grapes, treated by the studied pesticides groups, was carried out, in accordance with the methods [7]. According to these methods, the assigned points for the PDD of the pesticide, its stability in growing crops and the average daily consumption of apples and grapes, are added (Table IV). The summation of the obtained points for each criterion makes it possible to establish an integrated index of product safety (IIPS), and to establish a class of danger, according to this indicator.

The obtained results made it possible to establish, that most pesticides are moderately dangerous, according to the IIPS indicator, with the exception of kresoxim-methyl and clothianidin, which are not very dangerous (Table IV).

A comparative analysis of the parameters of pesticide resistance in the soil and climate conditions of Ukraine and EU countries showed no differences in pesticide hazard classes, regarding consumption of contaminated apples and grapes [9 - 11].

The obtained data on the resistance of pesticides in vegetative crops showed significant differences ($p\leq0.05$) in T50 for milbemectin, etofenprox, acetamiprid, novaluron, bifenthrin, clothianidin, lambda-cyhalothrin, during the fruit growth, in Ukraine compared to other countries. The specified differences can be caused by soil and climatic conditions, meteorological conditions during the growth of crops, as well as the application rate of the studied pesticides [9 - 11].

CONCLUSIONS

1. It has been proven that the processes of pesticide decomposition in vegetating agricultural crops (apples, grapes) occur, according to an exponential model (the coefficient of determination (R2) – 0.70-0.99).
2. Half-life period (T50, days) of the studied pesticides in agricultural plants was established: milbemectin (11.0 ±0.4), acetamiprid (11.2 ±0.2), novaluron (10.7 ±0.2), spirodiclofen (8.4 ±0 ,1), difenoconazole (11.7 ±0.1), fluxapiroxad (11.3 ±0.1), abamectin (12.1 ±0.2), glyphosate (7.7 ±0.7), kresoxim-methyl (8, 0 ±0.5), oxyfluorfen (5.7 ±1.3), clothianidin (7.9 ±0.2), lambda-cyhalothrin (7.6 ±0.1), etofenprox (8.4 ±0.9) and by stability belong to moderately resistant (class 3), bifenthrin (18.1 ±0.1) – resistant pesticides (hazard class 2), in accordance with DSanPiN 8.8.1.002-98 .
3. The calculated values of the risk of dangerous exposure to pesticides, when consuming apples and grapes, treated by them, were 2-3 orders of magnitude lower than the permissible level, and ranged from 2.0×10^{-3} to 7.8×10^{-2}.
4. It was shown, that according to the value of integrated index of product safety (IIPS), most pesticides are moderately dangerous (class 3), according to the indicator of IIPS, with the exception of kresoxim-methyl and clothianidin, which are slightly dangerous (class 4).
5. The obtained results should be taken into account, when considering the issue of expanding the field of use of pesticides, based on the investigated substances, and the necessity for conducting monitoring studies.

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ORIGINAL ARTICLE

CHANGES IN THE SENSORIMOTOR CORTEX OF THE RAT BRAIN UNDER THE MODELING OF HEMORRHAGIC STROKE

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ABSTRACT

The aim: To assess the structural and metabolic changes in the sensorimotor cortex of the rat brain under conditions of hemorrhagic stroke.

Materials and methods: The experiment was carried out on rats of the control and experimental groups with a model of hemorrhagic stroke. We used histological, electron microscopic, biochemical methods and biological markers.

Results: In the sensorimotor cortex of the ipsilateral cerebral hemisphere of rats under conditions of hemorrhagic stroke, cerebral edema and progression of neurodegenerative changes were observed; an increase in the size of mitochondria, which is caused by edema of their matrix; activation of lipid peroxidation processes and a decrease in the activity of enzymes of the antioxidant system, a decrease in the level of apoptosis markers and inhibition of ERK1/2 expression. The study of DNA fragmentation in the cerebral cortex revealed a significant number of manifestations of necrosis and an insignificant number of cells in a state of apoptosis.

Conclusions: after modelling a hemorrhagic stroke in the right hemisphere of the brain, perivascular and pericellular edema of the energy apparatus, cell death by necrosis and apoptosis, and activation of lipid peroxidation processes were established as well as a decrease in the activity of enzymes of the antioxidant system.

KEY WORDS: hemorrhagic stroke, ischemia, electron microscopy, biochemical changes, hypertension

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INTRODUCTION

Hemorrhagic stroke occurs due to hemorrhage in brain tissue after rupture of blood vessels and comprises about 15-20% of all strokes [1]. The common sites of hemorrhagic stroke are the basal ganglia (50%), cerebral lobes (10% to 20%), the thalamus (15%), pons and the brain stem (10% to 20%), as well as the cerebellum (10 %) [2]. In modern conditions, under the threat of Coronavirus disease to which the patients with major chronic diseases such as hypertension and diabetes are most vulnerable, these are also key risk factors for hemorrhagic stroke [3]. Thus, the study of morphological and biochemical changes, as well as biomarkers of apoptosis and necrosis will allow a deeper understanding of the mechanisms of hemorrhagic stroke and minimizing mortality and morbidity of patients in the future.

THE AIM

The aim of the study was to evaluate structural and metabolic changes in the sensorimotor cortex of the rat brain under conditions of hemorrhagic stroke.

MATERIALS AND METHODS

The experiment was performed on 30 rats, which were divided into 3 groups, the control was intact and pseudooperated rats, the differences between which were not detected, the experimental group - with a model of hemorrhagic stroke. Experiments with animals (weighing, measuring blood pressure, injections, operations) were carried out in accordance with the Law of Ukraine "On protection of animals from cruelty" (2006), "General ethical principles of animal experiments", adopted by the First National Congress on Bioethics (Kiev, 2001) and in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985).

Simulation of local hemorrhagic stroke in rats [4] was achieved by mechanical destruction of the inner capsule (SI dextra, L = 3.5-4.0; H = 6.0; AP = 0.6-1.0) by 4 -6 rotational movements of the bent mandrel-knife, followed by the introduction into the inner capsule of 0.15-0.2 ml of autologous blood. Ten days after stroke

simulation, intracardiac brain perfusion was performed with 4% paraformaldehyde solution on 0.1 N phosphate buffer (pH 7.4) and frontal sections of the motor area of the neocortex of the ipsilateral hemisphere [5] 2-3 mm thick were obtained.

In order to study histological changes, paraffin frontal sections of the brain [6] with a thickness of 6-8 μm were made on a Thermo Microm HM 360 microtome, stained with hematoxylin and eosin [7] and toluidine blue according to Nissl's method [8]. Morphometric and morphological analyzes were performed using Carl Zeiss software (AxioVision SE64 Rel.4.9.1) and Olympus BX 51 microscope (Japan).

In order to study the ultrastructural changes, fragments of the motor site of the neocortex of the ipsilateral hemisphere were removed and fixed in 2.5% solution of glutaraldehyde on phosphate buffer with fixation in 1% buffered saline of osmium tetroxide (OsO_4) and embedded into a mixture of epoxy resins epon-araldite (Epon 812, Araldite 502) [9]. Epoxy blocks were used to make semi-thin and ultra-thin sections on a Reihart ultratome. Ultrathin sections after contrast with 2% uranyl acetate solution were examined on an electron microscope Tescan Mira 3 LMU (Czech Republic).

THE STUDY OF DNA FRAGMENTATION OF CEREBRAL CORTEX FRAGMENTS

DNA was separated in 1.7% agarose gel (Agarose Serva Premium, "Serva", Germany) into fragments depending on molecular weight using the program "Gel Pro Analyzer". In agarose gel, apoptosis is defined as "DNA stairs" [10]. In necrosis, there is a rapid nonspecific cleavage of DNA into shorter fragments, which are registered as a "smear". In order to assess the activity of enzymes, determination of the amount of protein in the homogenate was performed by the method of Bradford [11]. The following monoclonal antibodies were used in Western bloting [12]: Bax, caspase-3 and ERK1/2 ("Cell Signalling", USA).

Catalase activity (CAT) was determined by the method of Aebi H. [13]. Superoxide dismutase (SOD) activity was determined by the Mirsa H. method [14]. NAD(P)H-quinone oxido-reductase activity was established in liver homogenates in a mixture of NADPH-generating glucose-6-phosphate dehydrogenase system, menadione (2-methyl-1,4-naphthoquinone) and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] [15]. LPOP products were detected by the method of determination of malonic dialdehyde (MDA) in the reaction with thiobarbituric acid (TBA) by the method of Uchiyama M [16]. The content of diene conjugates (DC) in the sample was calculated based on the molar coefficient of extinction at 233 nm for conjugated dienes of polyunsaturated higher fatty acids [17].

Statistical processing of the obtained data was performed using Student's t-test and Mann's U-test using Origin Lab (version 8.0) and Statistica 6.0 depending on the normality of the distribution.

RESULTS

Histological changes of the sensorimotor cortex of the cerebrum show (Fig. 1) neurodegenerative changes, namely edema of the cytoplasm and cell nuclei. Hyperchromic staining of neurons, deformation of nuclei and perikaryons, single intact gliocytes were determined. Altered lumen and necrosis of endothelial cells, concomitant perivascular edema with signs of infiltration of cells of monocytic-macrophage series are observed in capillaries.

The results of morphometric study confirm the progressive neurodegenerative changes in the sensorimotor cortex of the cerebrum of rats (Table I).

Gliocytes show signs of karyopicrosis as one of the morphological forms of apoptosis. The area of gliocyte nuclei in the experimental comparison groups decreased statistically significantly by 37.3% ($p < 0.05$).

Based on the results of electron microscopic examination, in the experimental group of rats with simulated hemorrhagic stroke significant dystrophic changes were found: severe edema of the neuropile and processes of neurons and gliocytes; a sharp decrease in the number of synapses. Only single interneuronal contacts were detected, and in some areas, they were completely destructed. The nuclei of neurons were swollen, the perinuclear space was increased, and the electron density of chromatin was reduced, which is a manifestation of fragmentation of nucleoprotein complexes. Nerve fibers were characterized by stratification of the lamellae of myelin sheaths (Fig. 2). Due to pronounced ultrastructural changes in cells, reduction of organelles and disruption of cell membrane integrity, we were unable to differentiate damaged small neurons from gliocytes, indicating the development of total neurodegenerative changes in the cerebral cortex during stroke, without significant differences between neurons and gliocytes.

Investigation of the energy apparatus of neurons - mitochondria**:** increase in the diameter of mitochondria (matrix and crystae edema of the organelles) was observed, which is a sign of edema of the cytoplasm of neurons. A statistically significant increase in the average diameter of mitochondria in experimental rats was determined – 342.5 ± 21.9 nm (control – 215 ± 41.2 nm). The increase in the size of mitochondria was due to edema of their matrix.

Analysis of biochemical changes in the integrated marker parameters of free radical oxidation of biomolecules showed a significant intensification of this process under the conditions of experimental stroke. In particular, this applies to the content of TBA-active products (MDA) and

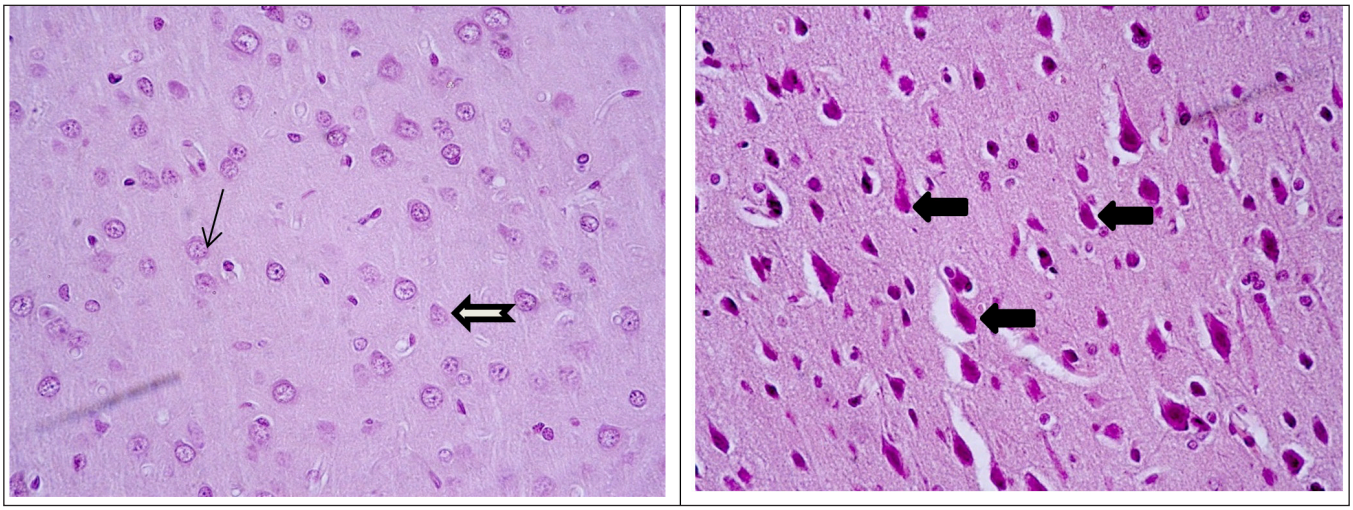

Fig. 1. Sensorimotor cortex of the cerebral hemisphere of rats with hemorrhagic stroke.
Note: ⬅ apoptotic neurons; ⇦ neurons in a state of hydropic dystrophy; neurons without changes; ← gliocytes. Hematoxylin-eosin. Obj. 40, oc. 10.

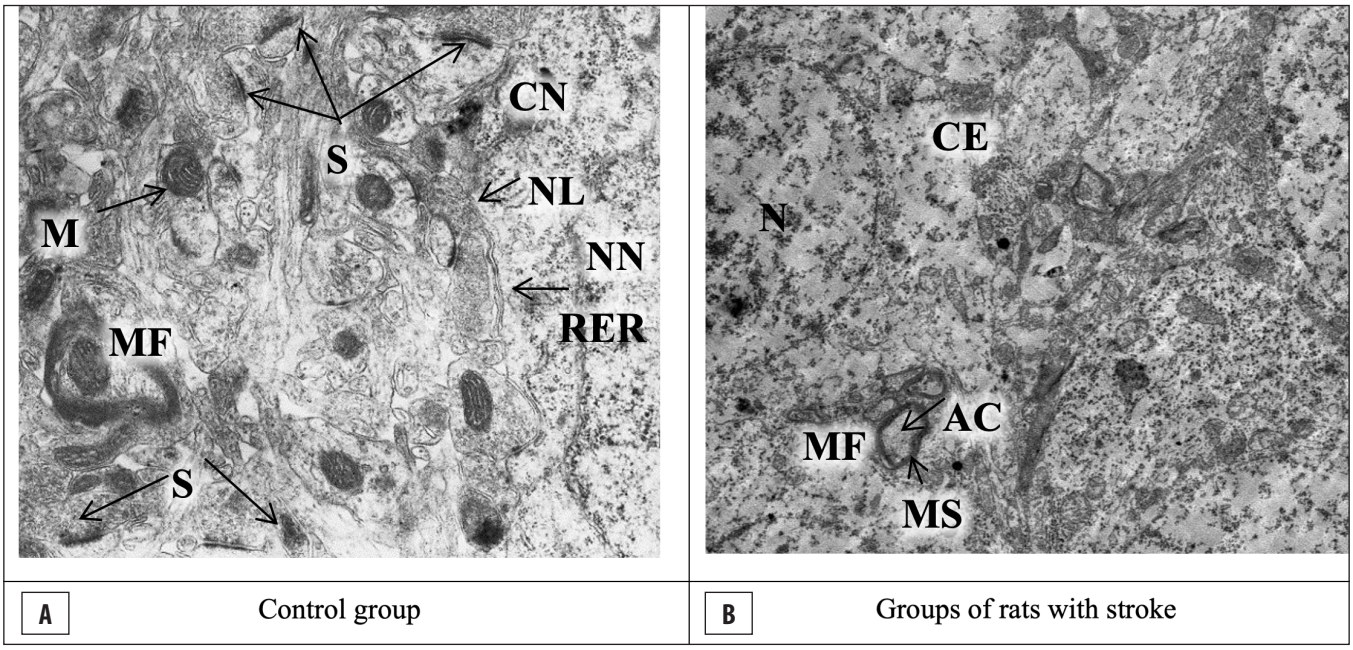

Fig. 2. Ultrastructural disorders in the cerebral cortex. A. Neuropil and neuron. Nerve cell processes are located densely. Synapses and single myelin fibers are registered. Note: NN is the nucleus of a neuron; NI - neurolemma; CN - cytoplasm of the neuron; MF - myelin fiber; S - synapse; M - mitochondria; RER - rough endoplasmic reticulum. Electronogram: × 25000.
B. Dystrophic changes of neuropil and neuron. Acute edema of the cytoplasm of the neuron with loss of integrity of membrane structures. Development of necrotic processes. Most myelin fibers have a detachment of the myelin sheath from the axial cylinder. Note: N – the nucleus of a nerve cell in a state of necrosis; CE - cytoplasmic edema; MF - myelin fiber; AC - axial cylinder; MS - myelin sheath. Electronogram: × 11400.

DC. It was found that the level of MDA in the homogenate of the cerebral cortex of normotensive rats was 2.2 times higher ($p < 0.01$) compared with the control group of rats, while DC was 1.5 times higher ($p < 0.01$) (Table II).

Our results showed that during stroke the activity of SOD and CAT decreased by 10.9% and 16.2% ($p < 0.05$) in rats with hemorrhagic stroke (Table II). According to the results of biochemical studies, it was found that in rats with hemorrhagic stroke, the activity of NAD (P) H-quinone oxidoreductase DT-diaphorase increased by 25.9% ($p < 0.05$) (Table II). Thus, under the conditions of hemorrhagic stroke, the activation of LPOP processes takes place, as evidenced by the increase in the content of LPOPs - MDA and DC products and the decrease in the activity of AES - SOD and CAT enzymes.

In the cells of the ipsilateral hemisphere of the cerebrum of rats on day 10 after simulation of hemorrhagic stroke, the dominance of necrosis over apoptosis was observed. This is accompanied by structural abnormalities in neurons that are associated with hyperproduction of endogenous cytotoxic molecules. The latter are formed as a result of

Table I. Morphometric parameters of sensorimotor cortex of rats on the background of modeled hemorrhagic stroke

Indicator / Group	Control	Experiment
Total density of neurons	104,7±3,9	99,4±5,1
Density of damaged neurons	5,0±0,9	39,9±4,3*
The area of the perikaryon of pyramidal neurons of the sensorimotor cortex, μm2	366,7±9,1	439,9±20,5*
The area of the nuclei of pyramidal neurons of the sensorimotor cortex, μm2	192,5±5,3	256,8±12,0*
The area of nuclei of gliocytes of the sensorimotor cortex, μm2	36,1±0,4	22,6±1,2*

Note: * - reliable to the control group (p <0,05).

Table II. The content of lipid peroxidation products in the cerebral cortex of control rats and rats with hemorrhagic stroke

Group of animals	Lipid peroxidation products(LPOPs)		Antioxidant Enzyme Systems (AES)		
	MDA, nmol/mg prot.	SOD U/mg/min prot	CAT mgmol/min prot	DT-diaphorase, nmol/mg/min	DC, nmol/mg prot.
Control	7,16±0,37	14,43±0,21	1,54±0,02	4,16±0,14	4,46±0,39
HS	15,54±1,05*	12,86±0,28*	1,29±0,05*	5,24±0,24*	6,93±0,87*

Note: HS - hemorrhagic stroke; * - changes are significant in relation to control (p <0,05).

oxidative stress in damaged mitochondria and cause the death of nerve cells. However, the decrease in the activity of the antioxidant system can be considered as a consequence of peroxidation of protein molecules of enzymes that perform the function of cell protection.

STUDY OF DNA FRAGMENTATION IN THE CEREBRAL CORTEX

DNA fragmentation was almost absent in control rats. DNA degradation was observed in the cerebral cortex tissue during hemorrhagic stroke, in the form of short DNA fragments (100-200 base pairs (bp)), which can be assessed as a manifestation of necrosis. However, on the electrophoregram we additionally found clear areas of DNA fragments divisible by 400 bp, which can be explained by the presence of a small number of cells in a state of apoptosis.

STUDY OF THE EXPRESSION LEVEL OF CASPASE-3 AND BAX IN THE CEREBRAL CORTEX

Quantitative analysis of the level of expression of caspase-3 indicates a decrease in its level compared to the level of its expression in control rats by 19.4% (p <0,05). In rats with hemorrhagic stroke, a similar pattern of reduction in the level of expression of Bax by 40.5% compared with controls (p <0.05) was found.

STUDY OF THE EXPRESSION LEVEL OF ERK1/2 IN THE CEREBRAL CORTEX

The expression level of ERK1/2 is lower in experimental rats compared to control rats. Inhibition of ERK1/2 expression can be considered as one of the mechanisms of cytoprotection.

DISCUSSION

Thus, the results of studies in the development of hemorrhagic stroke have shown cerebral edema and progression of degenerative changes neurons and capillaries, which confirms the morphological picture described by other researchers [4].

Electron microscopy revealed significant dystrophic changes in neurons and gliocytes, a sharp decrease in the synapses number, the absorption of which involves microglia/macrophages and astrocytes [18]. The observed damage of gliocytes is confirmed by new experimental data that even small changes in the structure of astrocytic processes in synapses can radically change the strength of synaptic transmission in the brain [19].

Neurons nuclei had manifestations of nucleoprotein complexes fragmentation, and nerve fibers were characterized by delamination of myelin sheaths lamellae, which was confirmed by own research results and data of other authors [20].

The study of mitochondria determined an increase in the diameter of mitochondria due to the swelling of their matrix. These data may explain the patterns of development of structural disorders on the background of stroke, namely the dominance of necrosis with underlying energy deficiency in cells and the development of cytopathological peroxidation of macromolecules (proteins, lipids, nucleic acids) and their subsequent decay. Mitochondrial dysfunction can lead to mitochondrial membrane potential collapse, overproduction of reactive oxygen species, disorders of

mitochondrial dynamics, and activation of mitochondria-related inflammation [21].

It was established that the level of MDA and DC in rats exceeded the indicators of the control group of rats. SOD and CAT decreased. A decrease in catalase activity leads to the formation of a hydroxyl radical, which causes lipid peroxidation. The activity of NAD(P)H-quinone oxidoreductase DT-diaphorase increased, which can be considered as a compensatory reaction of nerve cells to oxidative stress.

In the cortex of the ipsilateral hemisphere of rats with hemorrhagic stroke, cell death processes are observed, which are based on pronounced nonspecific fragmentation of deoxynucleoproteins, i.e. elimination of cells by necrosis. This assumption is confirmed by the data of other authors, who showed that in the condition of a stroke during the first hours the cells die by necrosis, and then the mechanisms of apoptosis join [22].

Signal pathways of apoptosis and necrosis initiation are often common, but the implementation of a certain mechanism of cell death depends on the characteristics of cell metabolism. Thus, it has been shown that ATP, reactive oxygen species (ROS), and nitrogen metabolites (NO, NO_2, $ONOO^-$) play an important role in switching between apoptosis and necrosis [23]. In addition, caspases and a number of other enzymes have been shown to play a key role in necrotic cell death. Thus, the decrease in the level of markers of apoptosis (caspase-3, Bax) and the dominance of total DNA fragmentation, i.e. nucleolysis indicate the progression of necrosis mechanisms over apoptosis. Simultaneous DNA fragmentation and activation of caspase-3 expression in the first 3 days of the experiment and activation of cytoprotective mechanisms on day 7 are known [24], which confirms the violation of apoptotic processes in the cerebral cortex. In our own studies, the tendency to decrease the synthesis of ERK1/2 indicates a violation of the mechanism of apoptosis activation, which may be interrelated with the development of necrosis and inhibition of regenerative processes in the cells of the cortex of large hemispheres in hemorrhagic stroke. This results in oligaemia, neuro-transmitter release, mitochondrial dysfunction, and cellular swelling [2].

CONCLUSIONS

After simulation of hemorrhagic stroke in the right hemisphere of the brain, perivascular and pericellular edema, dystrophic changes of neurons of III-V layers of the neocortex and cytoarchitectonics disorders as well as energy disorders, cell death by necrosis and apoptosis, activation of processes of peroxidation of lipids and reduction of activity of antioxidant system enzymes were observed.

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ORIGINAL ARTICLE

PHACE(S) SYNDROME - EARLY DIAGNOSTICS IN THE MAXILLOFACIAL AREA

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ABSTRACT

The aim: To determine the minimum criteria for early diagnosing PHACE(S) syndrome in neonates and infants with infantile hemangioma (IH) in the maxillofacial area.

Materials and methods: A total of 26 asymptomatic children from 20 days to six months of aged with IH of more than 5 cm^2 in the maxillofacial area were included in this study. A medical record of patients clinical examination, Holter monitoring, echocardiographic ultrasound and magnetic resonance imaging (MRI) were analysed. The IH treatment with ß-blockers was carried out.

Results: IH localization was diagnosed: 62% with a lesion of a part facial segment, 23% in one segment, 15% in several segments (p=0.018), and 12% with other parts of the body lesion (p=1.000). The patent foramen ovale was diagnosed in 35% of children. Central nervous system disorders were observed in 12% over two years of age. The indices of Holter monitoring and blood glucose changed in age norm range during treatment. Cardiovascular (the aortic coarctation (p=0.003) and brain (the Dandy-Walker malformation) (p=0.031) abnormalities were determined in two cases (8%) according to the MRI only. We diagnosed PHACE(S) syndrome in both these cases of children, only aged 12 months and 2.5 years old.

Conclusions: Early diagnosis of PHACE(S) syndrome is possible on a contrast-enhanced MRI performed in asymptomatic neonates and infants with the facial several segmental IH with / without ulceration (p=0.018, p=0.046; $p < 0.05$) for recognition of presymptomatic cardiovascular and brain abnormalities.

KEY WORDS: aortic coarctation, infantile hemangioma, PHACE(S) syndrome, ß-blockers, magnetic resonance imaging

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INTRODUCTION

PHACE(S) is a neurocutaneous disorder of unknown etiology. In 1996, Frieden et al. suggested the abbreviation PHACE(S)–(P) posterior fossa malformations, (H) infantile hemangioma, (A) arterial anomalies, (C) coarctation of the aorta/cardiac defects, (E) eye abnormalities and ± (S) sternal malformations) [1]. PHACE(S) syndrome has a higher risk of developing in children with infantile hemangioma (IH) occupying even a single facial segment, with a frequency of 20–31% in this group [2]. PHACE(S) syndrome is characterized by a combination of symptoms that may not be immediately apparent at birth. Timely diagnosis and interdisciplinary treatment of patients with PHACE(S) syndrome are crucial, considering the clinical polymorphism of this syndrome and the difficulty in identifying symptoms. Importantly, this can prevent the development of complications, such as cardiovascular collapse, thromboembolism, stroke, paralysis, loss of vision, moyamoya disease and hypopituitarism [3,4]. The clinical manifestations of cardiovascular system (CVS) diseases are especially characterized by slow progression. Therefore, the visibility of clinical signs depends on their severity.

Our hypothesis was that clinical symptoms of PHACE(S) syndrome can be not clear in neonates and infants with a facial IH and leveled against the background of taking ß-blockers in children with the syndrome, which makes it difficult to set up.

THE AIM

Therefore, the present study was undertaken to determine the minimum criteria for early diagnosing PHACE(S) syndrome in neonates and infants with IH in the maxillofacial area (MFA).

Working null hypothesis: 1) There is no relationship between PHACE(S) syndrome and clinical symptoms. 2) The drug therapy has no effect on indices of heart rate, AP, indices of cardiac rhythm variability and blood glucose level.

MATERIALS AND METHODS

This case-control study was performed at the surgical dentistry and maxillofacial surgery of childhood department. The object of the study were 26 children aged from 20 days to six months old (at the time of admission) with a IH. The inclusion criteria were: (1) with a IH of more than 5 cm^2 in the MFA; (2) no CVS and neurological signs on a first clinical examination before the time of admission; (3) any treatment was not carried out; and (4) clinical follow-up available until at least the age of 3 years for asymptomatic children. Patients were excluded if they had: (1) a IH of less than 5 cm^2 in the MFA; (2) unable to co-operate with follow-up. All children were examined and observed by pediatricians, cardiologists, oculists, ENT doctors and neurologists. Clinical examination involved medical record of patients examination (Table I), doppler ultrasound of IH, Holter monitoring, echocardiographic ultrasound for all patients. A contrast-enhanced magnetic resonance imaging (MRI) was performed in four children with IH localization in several segments; parents did not consent to the study in other cases. The patients took propranolol for IH in the dose of 2 mg/kg three times a day from 20 days to two years, depending on the decrease in IH volume and vessel number. Side effects and the dose of the drug were controlled in accordance with monitoring blood glucose level, arterial blood pressure (AP) and Holter monitoring (heart rate, indices of cardiac rhythm variability (CRV)). Data obtained within before treatment, at the point of prescribing the drug and second increasing dose were used to calculate statistical (Table II).

All procedures and studies were performed after obtaining informed consent from the parents of participants. The study was reviewed and approved by the research committee.

STATISTICAL ANALYSIS

Descriptive statistics were used to analyse the characteristics of the study population. To determine test performance characteristics, the results were illustrated in a cross tabulation according to the anamnestic and clinical data diagnosis of PHACE(S) syndrome. The groups were compared by the Fisher's exact test, the level of significance was set at $p < 0.05$. The Friedman test for repeated measures were applied to compare the indices of heart rate, AP, blood glucose and CRV ($p < 0.05$). Post hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied, resulting in a significance level set at $p < 0.017$. Statistical data processing was performed through IBM SPSS Statistics 29.0.1.0(171).

RESULTS

An analysis of 26 case histories of children, 20 girls and six boys, with IH of MFA was carried out. Pregnancy anamnestic screening of the children`s mothers showed that it was physiological in 27% of cases, early pregnancy toxemia in 42.3%, pre-eclampsia in 19.2% and risk of miscarriage in 11.5% (Table I). Two babies were born prematurely (< 37 gestation age at birth), and four were born with low birth weight (< 2900g), two of which were from a twin pregnancy. Fetal hypoxia was corrected in seven cases, connected with cord entanglement, respiratory failure or twin pregnancy. In all cases, IH manifested immediately after birth or in the first week of life. Localization was diagnosed: 62% with a partial lesion of a facial segment, 23% in one segment, 15% in several segments, and 12% with IH combined with back, limb, or liver damage. Active growth was noted in all cases. The IH had an area of 5 cm^2 to 20 cm^2 in 65% of cases and over 20 cm^2 in 35% of cases. It appeared on the skin as nodules and purple spots with clear contours in 54% of patients, with solid red spot in 42%, was accompanied by tissue deformation with a slight vascular pattern in 42%, a loose vascular network in 4%, and ulceration of IH sites was noted in 23%. Doppler ultrasound was performed in all patients to confirm the diagnosis of IH and the need for future medical treatment monitoring. Blood flow velocity was within 42.8-90.4 cm/s. (55.7 ± 5.2 cm/s.) in the arterial vessels and 5.3-15.4 cm/s. (10.27 ± 1.02 cm/s.) in the venous vessels.

Any parents of patients had not complaints of CVS. As a result of determining cardiovascular profile patent foramen ovale (PFO) was diagnosed in 35% of children during heart ultrasound. No other CVS changes on echocardiographic ultrasound were observed during the initial examination. After examination and diagnosis, treatment with propranolol in the appropriate weight-based dosage commenced. The median heart rate before treatment was 138.0 ± 1.6 beats per minute, according to the Holter monitoring data. The minimum heart rate value, observed at night, ranged from 99 to 134 beats per minute prior to the start of treatment and from 72 to 107 beats per minute during treatment. The heart rate of all children decreased by a factor of 20% while taking the standard dose at the beginning of treatment. The heart rate then decreased by a factor of 30% with the second increasing dose therapy in relation to the heart rate before treatment. The result turned out to be statistically significant ($p<0.001$) for all pairs of comparison groups (Table II). The correlation of the CRV indicators was fixed to evaluate its low and high-frequency components - SDNN, rMSSD, and SDANN. We established that CRV indicators that changed during propranolol therapy were not signifi-

Table I. Medical record of patients examination with infantile hemangioma in the maxillofacial area

Characteristics	n = 26		Statistical significance (p)*
	No syndrome n = 24 (%)	PHACE(S) syndrome n = 2 (%)	
Sex			
Female	18 (69)	2 (8)	1.000
Male	6 (23)	-	
Gestational age at birth			
Full term (≥37 wk)	22 (84)	2 (8)	1.000
Premature (≥32 to <37 wk)	2 (8)	-	
Birth weight			
Norm (≥2900g to < 3900g)	20 (77)	2 (8)	1.000
Low (< 2900g)	4 (15)	-	
Pregnancy			
Physiological	7 (27)	-	
Early toxemia	9 (35)	2 (8)	1.000
Pre-eclampsia	5 (19)	-	
Risk of miscarriage	3 (11)	-	
Fetal hypoxia			
No hypoxia	17(65)	2 (8)	
Twin pregnancy	2 (8)	-	1.000
Cord entanglement	4 (15)	-	
Respiratory failure	1 (4)	-	
IH localization			
Local	16 (61)	-	
Segmental	6 (23)	-	0.018
Multiple segments	2 (8)	2 (8)	
Combination IH of a facial with other parts of the body	3 (12)	-	1.000
Tissue change in the IH affected area			
Loose vascular network	-	1 (4)	
Solid red spot	10 (38)	1 (4)	0.034
Red spots and nodes presence	14 (54)	-	
Tissue deformation presence	10 (38)	1 (4)	1.000
No tissue deformation	14 (54)	1 (4)	
IH ulceration	4 (15)	2 (8)	0.046
IH measurements			
≥5 см ^{2} to <20 см ^{2}	17 (65)	-	0.111
≥20см^2	7 (27)	2 (8)	
Changes by the CVS			
No pathology	15 (57)	-	
Patent foramen ovale	9 (35)	-	0.003
Aortic coarctation	-	2 (8)	
Changes by the central nervous system			
Anomalies of cerebral vessels	-	1(4)	
Headache	1(4)	2 (8)	0.031
Dandy-Walker malformation	-	1(4)	
Convulsive disorder	2 (8)	-	
No pathology	21 (80)		

* Fisher's exact test, the level of significance was set at $p < 0.05$.

cant ($p > 0.05$). Isolated cases of II grade type I sinoatrial block were reported in 42% of cases, both before and during treatment, but their presence corresponded to the age norm. Blood pressure decreased during treatment, a factor of 10% for systolic and a factor of 20% for diastolic, but were changed in age norm range.

Table II. Changes in examination parameters during treatment with propranolol

Examination parameters	Before treatment Me ± m	Start of treatment Me ± m	The second increasing dose therapy Me ± m	Statistical significance (x^2 (df), p) *
Heart rate (beats *per* minute)	138.0 ± 1.6	110.0 ± 1.0	97.0 ± 0.5	$x^2(2) = 52.000$, < 0.001
Systolic AP (mm Hg)	102.5 ± 2.6	94.5± 2.2	89.5± 2.0	$x^2(2) = 48.080$, < 0.001
Diastolic AP (mm Hg)	62.0 ± 1.6	55.0 ± 1.2	50.0 ± 0.7	$x^2(2) = 51.515$, < 0.001
Blood glucose (mmol/l)	4.8 ± 0.1	4.65± 0.06	4.50± 0.05	$x^2(2) = 17{,}383$, < 0.001

* The Friedman test was conducted (95% CI) with a Bonferroni correction applied, resulting in a significance level set at $p < 0.017$

There was a statistically significant AP reduction in during comparisons between with each of the groups ($p < 0.001$) depending on the drug dose rise. Glucose testing, standard in ß-blocker therapy, was also carried out on all children. The blood glucose level varied within the normal range of 4.65± 0.06 mmol/l during the entire treatment period. Pairwise comparisons of changes in blood glucose parameters revealed a statistically significant difference only between "Before treatment" and "Second increasing dose therapy" ($p < 0.001$) (Table II).

Central nervous system (CNS) disorders were observed as manifestations of convulsive disorders in 8% of children with IH. There were complaints of headaches in 12% of children over two years of age.

An MRI study was performed in four children with IH localization in several segments. Brain structure and blood vessel changes were determined according to the MRI only in two cases (8%). The aortic coarctation was diagnosed, with hypoplasia of the left cerebellar hemisphere as a variant of the Dandy-Walker malformation found in one child of 12 months. The aortic coarctation did not manifest itself clinically in the second child of 2.5 years old, so was diagnosed with a repeat echocardiographic ultrasound. This child underwent an MRI that confirmed aortic coarctation and anterior cerebral artery A1 segment stenosis on the right. The right posterior communicating artery hypoplasia and the circle of Willis disjoin were also detected. An MRI was performed in the same child at six months, where blood vessels changes did not reveal. The results of the Fisher's exact test indicate a significant association between "Syndrome" and "Characteristics" for: multiple segments IH localization, vascular pattern character, ulceration, aortic coarctation and CNS changes (Table I). The level of significance was set at $p < 0.05$.

We diagnosed PHACE(S) syndrome in both these cases of children, only aged 12 months and 2.5 years old only after MRI was performed. The coarctation was surgically repair after diagnosed.

DISCUSSION

ANAMNESTIC SCREENING

According to the study using the PHACE(S) Syndrome International Clinical Registry and Genetic Repository, this syndrome develops (up to 80%) in premature babies of less than 37 weeks of gestation and miscarriages in the first trimester of pregnancy. The results also show that preeclampsia, placenta anomalies, and injuries and hemorrhages can be potential prenatal risk factors (up to 15%) for PHACE(S) syndrome. These symptoms are much more prevalent in this group than in patients with IH [5]. Due to all these factors, mothers of children with the syndrome have been observed to have only toxicosis in the first trimester of pregnancy in our study.

CLINICAL SCREENING

In 2016, new recommendations on diagnostics and successive observation of patients with PHACE(S) syndrome were published by an interdisciplinary group [4]. One of the principal criteria of the syndrome is IH within the MFA. The studies carried out by Haggstrom A.N., (2010) showed that the IH area of the face constituted 22 cm^2 and over (~ 5 cm × 4.5 cm) in 31% of infants meeting the diagnostic criteria of the syndrome [2]. However, literature has been known to describe cases of PHACE(S) development in minor, non-segmental afflictions located in the limb area and with no dermal IH [6]. Consequently, PHACE(S) syndrome is determined in the final protocol by the presence of segment IH exceeding 5 cm^2 in the face, head, or upper part of the trunk, in addition to one or two other basic criteria [4]. The patients with PHACE(S) syndrome under our surveillance were originally diagnosed to only have IH in the MFA. Nevertheless, in one child, the area extended to three segments (frontonasal, frontotemporal and maxillary) with ulceration of the upper lip. In another child, all segments were affected bilaterally, with the

left orbit exhibiting and some ulceration within the area of auricles.

SOMATIC CONCOMITANT DISORDERS OF THE CVS AND CNS

In different sources, the frequency of cardiovascular disorders in PHACE(S) varies within the range of 21-67%, greatly exceeding the prevalence in the general population and other syndromes linked to congenital heart defects (such as Turner syndrome) [2- 4]. According to the International Registry of PHACE(S) Syndrome, 41% of patients have heart, aorta or brachycephalic vessel anomalies. Severe cases usually become evident shortly after birth. A child can abruptly become pale, irritable, diaphoresis excessively, breathless, and suffer from vomiting unrelated to food intake. The given signs had not been noticed by the parents of the children in our study with PHACE(S) syndrome. Aortic coarctation ranks the second (19%), and can be clinically overlooked in patients with PHACE(S) syndrome due to repeated associations with arch obstruction of aberrant subclavicular origin [4,7]. The children under our observance complained of headaches - mainly those over two years of age. One child had a single episode of nasal bleeding that could have been connected with the raised AP. Clinical symptoms depended on the degree of aortic stenosis. Patients with severe narrowing were demonstrated to have such symptoms in childhood, while minor constriction may not produce any changes in the body for a prolonged period of time. The principal technique used to confirm the disease is echocardiographic ultrasound; however, the frequency of revealing aortic coarctation using trans-abdominal access is less than 56% [8, 9]. Therefore, it is not considered to be a reliable way as well. Aortic coarctation was not established in our cases when employing echocardiographic investigation on the initial stages of diagnostics. ß-blockers, particularly propranolol, are a beneficial first step in managing IH, whenever systemic therapy is indicated [10-12]. As we treated the children with ß-blockers beginning 20 days - six months, the preliminary clinical picture smoothed out due to the main effect of the drug–reduction of heart rate and AP. Consequently, clinical symptoms characteristic of cardiovascular disturbances were not revealed; on the contrary, bradycardia and AP reduction in age norm range were found to be present.

High rates of cerebrovascular abnormalities (91%) and structural afflictions of the brain (42%) are characteristic of PHACE(S) syndrome [13, 14]. Narrowing or absence of visibility of the principal cerebral or cervical arteries is considered one of the core criteria for the diagnosis of MRA, which was diagnosed only in the 2.5-year-old patient. The spectrum of posterior cranial fossa abnormalities, from the focal afflictions of the cerebellum to Dandy-Walker complex, in patients with PHACE(S) varies from 30.4% to 81% in described cases [13]. The most typical clinical symptom resulting from these changes involved headaches (55.3%). The average age of children was four years, with a range of 12 months to 10 years [15, 16].

ENDOCRINE ABNORMALITIES

Growth hormone deficiency is more commonly recognized as a characteristic one in PHACE(S) syndrome. The majority of the cases informed are linked with thyroid dysfunction and hypopituitarism having unechogenic or hypoechogenic sella turcica or structural abnormalities of the pituitary gland seen on the MRI. Neonatal hypoglycemia can be a sign of hypopituitarism [17]. These signs have not been proved to be seen in our patients.

Our study has several limitations. The number of children included was limited by the low prevalence of the disease. Our clinical experience has shown that diagnosing PHACE(S) syndrome is complicated by the inability to obtain sufficient complaints, take anamnesis from parents and vague clinical symptoms. Except for IH, not all clinical signs are present at birth and usually develop with age. The interdisciplinary group recommends that additional diagnostic techniques, such as MRI, should administered in the presence of clinical symptoms suggestive of PHACE(S) syndrome [4]. So haw age should an MRI be done to make for early diagnosing PHACE(S) syndrome? When does advisable to repeated the study in asymptomatic children? The clinical picture of the syndrome depends on the extent of expression of the CVS and CNS disorders. Moreover, the possibility of syndrome progression should be considered if IH occurs on the other parts of the body in addition to the face. Clinical signs of CVS and CNS diseases not related to PHACE(S) syndrome might be indicative of a separate ailment. All symptoms must be present in combination to verify the syndrome, although connecting a specific symptom to PHACE(S) syndrome in young children can be challenging.

CONCLUSIONS

Early diagnosis of PHACE(S) syndrome is possible on a contrast-enhanced MRI performed a patient meets the clinical diagnostic criteria – IH of the MFA in several segments without/with ulceration ($p=0.018$, $p=0.046$; $p < 0.05$). A contrast-enhanced MRI monitoring expression of the head, neck and aortic arch should be performed,

along with ultrasound scanning of the heart, as a part of the primary diagnosis of PHACE(S) syndrome in asymptomatic neonates and infants with several segments IH of the face. Recognition of presymptomatic cardiovascular abnormalities, anomalies of the brain involvement is essential to identify the population at risk of cardiovascular, neurological complications for the prognosis and organizing subsequent medical follow-up.

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ORIGINAL ARTICLE

ANALYSIS OF PHOTOMETRIC FULL-FACE PARAMETERS WITH A DISTAL BITE TAKING INTO ACCOUNT THE TYPES OF MANDIBULAR GROWTH

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ABSTRACT

The aim: Determination of the peculiarities of full-face aesthetics in patients aged 10-13 years with a distal bite with different types of mandibular growth.

Materials and methods: 74 patients aged 10-13 years with a distal bite and various types of mandibular growth took part in the study. The methods of photostatic image study described by I.I. Uzhumetskene (1970), V.P. Pereverzev (1979) were used in this research.

Results: To determine the type of face, a gl-me: zy-zy index was calculated. Index values ranging from 0.84 to 0.879 are indicative of a mesoprosopic (medium) type of face, an index less than 0.839 defines a europrosopic (broad) type, an index exceeding 0.88 is characteristic of a leptoprosopic (narrow) type. The facial shape in all patients was determined by the Izar facial-morphological index. Similarly, the facial shape can be determined by the facial tapered angle (< FTA). In all groups of teenagers, a decrease in the angle is noted, which is indicative of an elongated, narrow face. To assess the ratio of the middle and lower parts of the face, the gl-sn:sn-me index was used, which is normally equal to 1. In our case, this index was higher than normal in all groups of children as compared to the control group (1.01±0.03). The p-p:zy-zy and p-p:go-go indices are indicative of the harmonious facial development transversally.

Conclusions: Vertical and neutral-vertical type of mandibular growth results in a narrow (leptoprosopic) type of face; medium (mesoprosopic) and wide (europrosopic) types of face result from neutral, horizontal and neutral-horizontal types of growth. Irrespective of the type of mandibular growth, all patients with a distal bite have a short lower third of the face. The strongest aesthetic changes are reported in the group of patients with a predominance of the horizontal type of mandibular growth.

KEY WORDS: distal bite, photometry, full-face, type of growth

Wiad Lek. 2023;76(9):2028-2033

INTRODUCTION

A persistent tendency of modern orthodontics to shift the diagnostic priorities from the hard tissues of the skeleton to the soft tissues of the face and identification of their correlation is connected with the growing aesthetic demand among the patients and the development of technologies that allow satisfying this demand [1, 2]. The primary task of an orthodontist is to restore the facial proportions, create an aesthetic half-face, full-face and a beautiful natural smile.

Distal bite is characterized by specific facial features, namely: a convex type of face, a tilted back chin, a short lower third of the face, a deep chin-lip fold, lips disproportion [3-6]. Orthodontic treatment of patients with this anomaly lead to significant changes in appearance, which may not meet the standards of aesthetic optimum if the chosen therapeutic approach is wrong [7].

The scientific orthodontic literature contains numerous methods of soft tissue analysis; however, the planning of facial attractiveness is still quite difficult in the treatment of distal bite.

For the purposes of face study, various measurements and identification of changes caused with the treatment, the method of full-face and half-face photometry of the patients is widely used in orthodontics.

We did not find any data in the scientific references available that would discover the connection between full-face photometric studies in patients with a distal bite and the type of their mandibular growth.

THE AIM

Determination of the peculiarities of full-face aesthetics in patients aged 10-13 years with a distal bite with different types of mandibular growth.

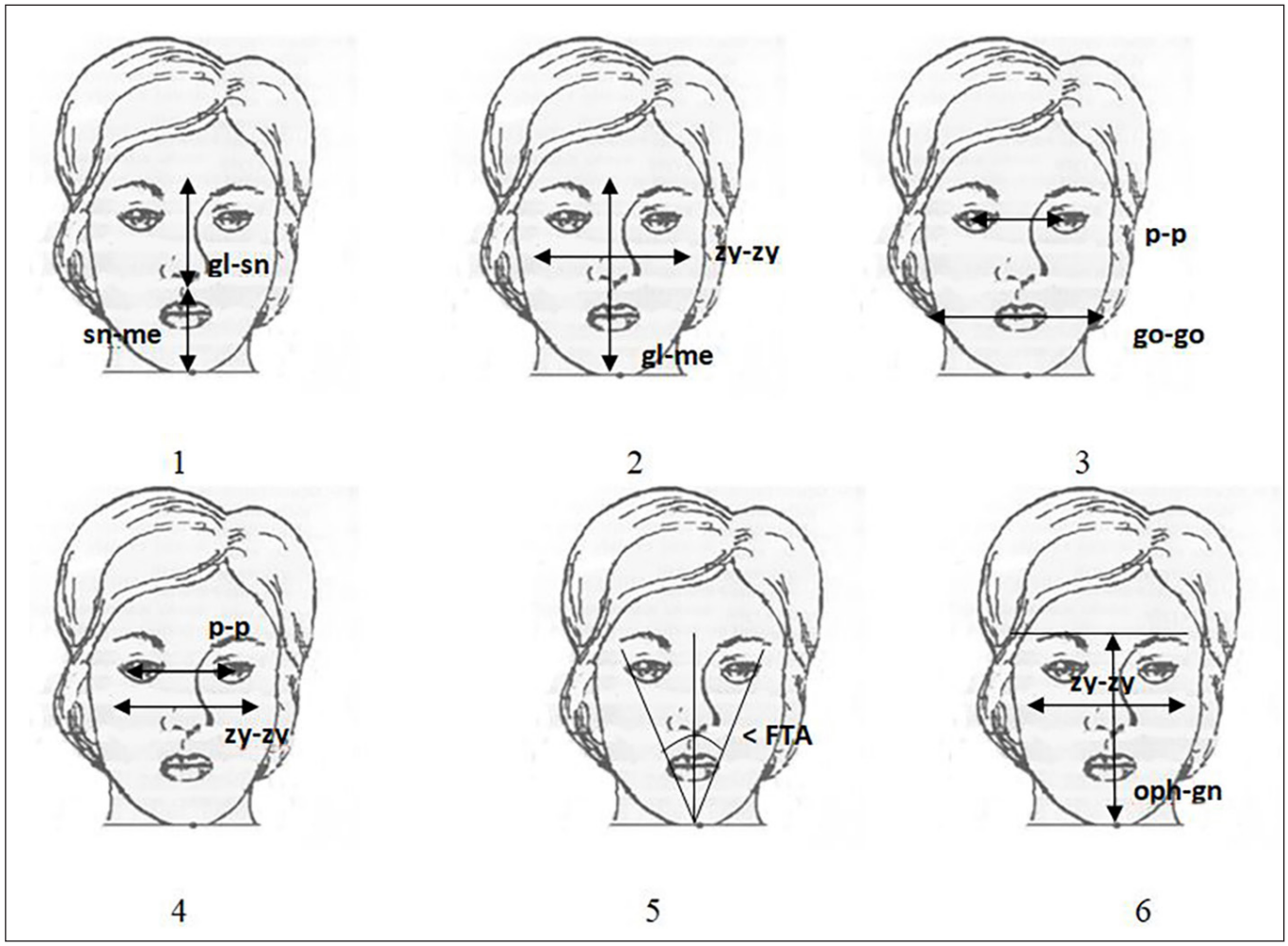

Fig. 1. Determination of full-face parameters: 1 - gl-sn: sn-me index (N = 1); 2 - gl-me: zy-zy index (N = 0,85); 3 - p-p: go-go index (N = 0,5); 4 - p-p: zy-zy index (N = 0,75); 5 – facial tapered angle (N = 45±5°); 6 - Izar facial and morphological index (N = 97-103).

MATERIALS AND METHODS

74 patients aged 10-13 years with a distal bite and 27 patients of the control group with abnormal position of individual teeth took part in the study. Patients were divided into five groups taking into account the type of mandibular growth determined with orthopantomograms according to the method proposed by the German researchers. The first group consisted of 28 patients with a neutral type of mandibular growth; the second group consisted of 13 patients with a vertical type; the third group included 11 patients with a horizontal type; the fourth and fifth groups were represented by children with a combined type of mandibular growth, of which 12 children had neutral-vertical type and 10 children had neutral-horizontal type of mandibular growth.

The study of facial aesthetics in children with a distal bite was carried out using photometry. For this, we applied the methods of studying full-face photostatic images of faces, which were described by T.F. Kosyreva (1996).

When assessing the full-face, the gl-sn: sn-me, gl-me: zy-zy, p-p: go-go, p-p: zy-zy indices and the facial tapered angle were determined. The Izar facial and morphological index was also determined (Fig. 1).

The results were processed statistically using the Student-Fischer test ($p \leq 0.05$).

RESULTS

The results of full-face photometric study of patients with neutral, vertical, horizontal and combined types of mandibular growth are presented in Table I.

To determine the type of face, the gl-me: zy-zy index was calculated (Fig. 2). Index values ranging from 0.84 to 0.879 are indicative of a mesoprosopic (medium) type of face, an index less than 0.839 defines a europrosopic (broad) type, an index exceeding 0.88 is characteristic of a leptoprosopic (narrow) type. The study determined that the average value of the gl-me:zy-zy index in the control group, i.e. in children with abnormal position of individual teeth, was 0.86±0.01. In patients with a

Table I. Characteristics of full-face photometry indicators in patients aged 10-13 years with a distal bite

Parameter	Control group n=27	Types of mandibular growth									
		Neutral n=28	P	Vertical n=13	P	Horizontal n=11	P	Neutral-vertical n=12	P	Neutral-horizontal n=10	P
gl-sn:sn-me index	1.01±0.03	1.15±0.02	<99.9	1.08±0.05	<95	1.19±0.02	99.9	1.05±0.02	<95	1.14±0.05	95
gl-me:zy-zy index	0.86±0.01	0.84±0.01	95	0.93±0.02	99	0.84±0.02	<95	0.89±0.01	99	0.84±0.02	<95
p-p:go-go index	0.51±0.01	0.58±0.01	99.9	0.57±0.01	99.9	0.59±0.01	99.9	0.55±0.02	<95	0.54±0.03	<95
p-p:zy-zy index	0.75±0.01	0.47±0.01	99.9	0.47±0.01	99.9	0.48±0.01	99.9	0.48±0.02	99.9	0.45±0.01	99.9
FMI by Izar	98.57±0.86	83.64±0.85	99.9	107.08±1.14	99.9	79.91±0.74	99.9	108.17±1.42	99.9	80.3±0.33	99.9
< FTA	42.68±0.65	37.21±0.6	99.9	36.15±1.02	99.9	39.18±1.26	>95	36.75±0.34	>99.9	38.1±0.72	99.9

vertical direction of mandibular growth, this index was higher (0.93±0.02 for vertical type of growth, 0.89±0.01 for neutral-vertical growth), which corresponds to a leptoprosopic (narrow) type of face. Concurrently, in children with neutral, horizontal and neutral-horizontal types of growth, the values of the index tend to decrease and border on mesoprosopic (medium) and europrosopic (wide) facial types (0.84±0.01, 0.84) ±0.02, 0.84±0.02, respectively).

The facial shape in all patients was determined by the Izar facial-morphological index (Fig. 3). This index is 98.57±0.86 in the control group. The statistically significant value of the facial morphological index in patients with neutral, horizontal and combined neutral-horizontal types of mandibular growth did not exceed 86 (83.64±0.85; 79.91±0.74; 80.3±0.33 respectively). This factor is indicatives of a wide face in children with the specified types of growth. The patients with vertical and neutral-vertical types of growth can be defined as having a narrow face with the Izar facial-morphological index 107.08±1.14 and 108.17±1.44, respectively.

Similarly, the facial shape can be determined by the facial tapered angle (< FTA) made by two lines connecting the lateral points of the eye sockets with the corners of the mouth (Fig. 4). The angle value in the control group is 42.68±0.65° on average; however, a decrease in the angle value is reported in all groups of teenagers, which is indicative of an elongated narrow face. The greatest difference in the downward direction of this indicator is established in the group with vertical and neutral-vertical types of mandibular growth, with 6.53 and 5.93 on average, respectively. In patients with neutral, horizontal and neutral-horizontal types of growth, this indicator was closer to the control group (medium facial shape), but peculiar to a narrow face (37.21±0.6; 39.18±1.26; 38.1±0.72, respectively). The obtained results somewhat do not correspond to the given previous parameters, and they are unlikely to be unconditional for the purposes of the facial shape determination in patients with a distal bite.

To assess the ratio of the middle and lower parts of the face, the gl-sn:sn-me index was used, which is normally equal to 1 (Fig. 5). If a face is harmonious, both parts should be equal. In our case, in all groups of children, the index was higher than normal if compared to the control group (1.01±0.03). The highest values of the index are reported in groups of patients with neutral, horizontal and neutral-horizontal types of the mandibular growth (1.15±0.02, 1.19±0.02 and 1.14±0.05, respectively). In case of vertical and neutral-vertical types of growth, this indicator is significantly lower as compared to the above-mentioned groups (1.08±0.05 and 1.05±0.02, respectively), but higher than normal. The obtained data are indicative of a short lower part of the face compared to the average, which is not harmonious.

The harmonic development of the face transversally is indicated by the p-p:zy-zy and p-p:go-go indices, which normally are equal to 0.75 and 0.5, respectively (Fig. 6). In all studied groups, regardless of the type of mandibular growth, a decrease in the p-p:zy-zy index was reported from 0.48±0.01 with horizontal and neutral-vertical growth to 0.45±0.01 with neutral-horizontal growth.

In case of neutral-horizontal and horizontal types of growth, the p-p:go-go index shows a statistically significant increase in all patients from 0.54±0.03 to 0.59±0.01, respectively. Thus, the facial aesthetics of patients with a distal bite cannot be considered harmonious.

DISCUSSION

Specific facial features are observed in patients with a distal bite [3-5]. In the course of orthodontic treatment of patients with the mentioned anomaly, significant changes are observed in their appearance [6, 7].

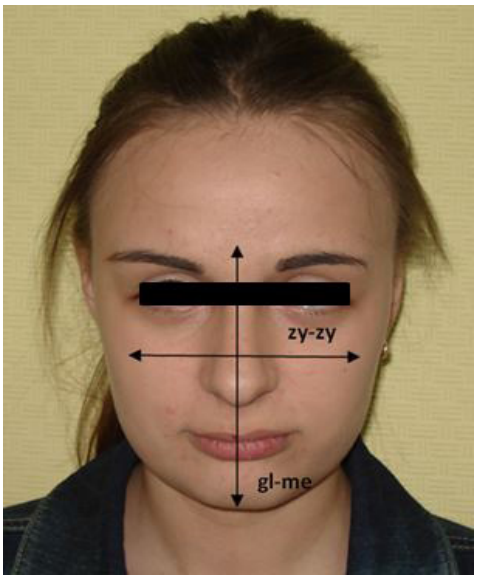

Fig. 2. Patient A., aged 13, neutral type of mandibular growth, gl-me:zy-zy index =0.95

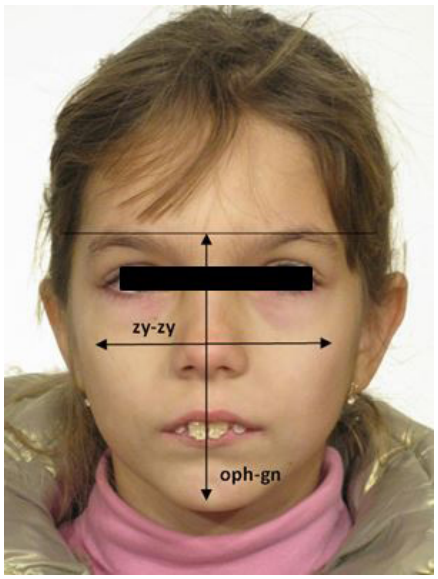

Fig. 3. Patient A., aged 10, vertical type of mandibular growth, Izar facial and morphological index = 1.17.

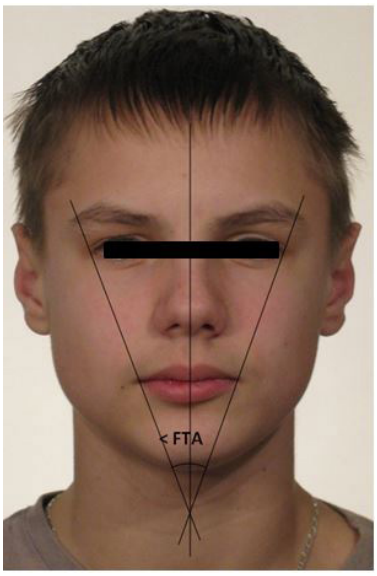

Fig. 4. Patient A., aged 12, horizontal type of mandibular growth, facial tapered angle (< FTA) = 40°.

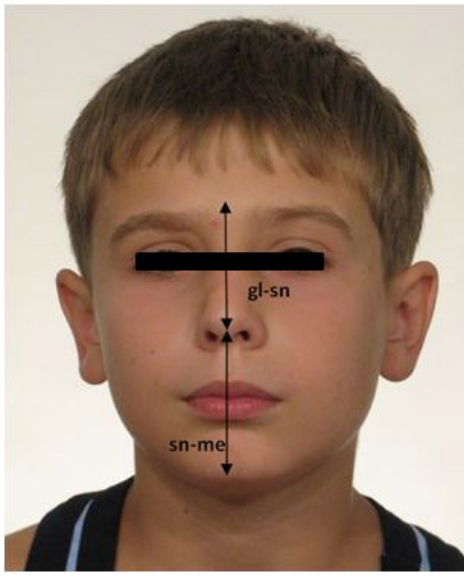

Fig. 5. Patient A., aged 10, neutral-vertical type of mandibular growth, gl-sn:sn-me index = 1.0.

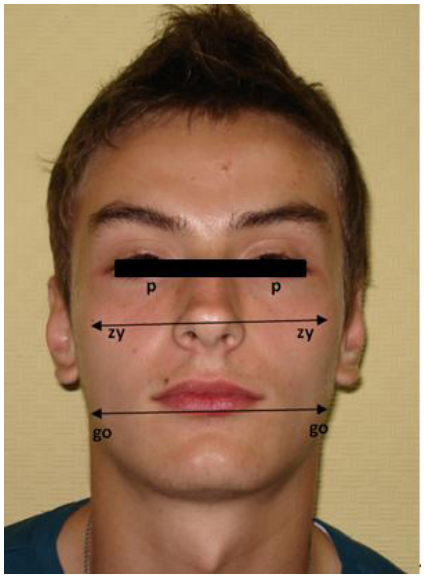

Fig. 6. Patient O., aged 13, neutral-horizontal type of mandibular growth, p-p:zy-zy index = 0.57 and p-p:go-go index = 0.51.

One of the informative diagnostic methods used to determine a orthodontic approach and a treatment plan for patients with a distal bite is photometry [1, 2]. However, we did not find any information in the domestic and foreign scientific orthodontic literature about full-face photometric studies in patients with the above-mentioned pathology of the bite, depending on the types of mandibular growth.

Facial photometry of 74 patients aged 10-13 years with a distal bite was compared with 27 patients with abnormal position of individual teeth.

The type or shape of the face was studied using the gl-me:zy-zy index. In patients with a vertical direction of mandibular growth, its increase was found in the case of a vertical direction (p=0.01) and in a neutral-vertical direction (p=0.01), which corresponds to a leptoprosopic (narrow) type of face. Meanwhile, in children with neutral, horizontal and neutral-horizontal types of growth, this index was reported to decrease and reach the boundary between mesoprosopic (medium) and europrosopic (wide) types of face.

Also, the facial shape was determined with Izar facial-morphological index, which is 98.57±0.86 in the control group. The high statistically guaranteed value of the facial-morphological index (p=0.001) shows that

patients with neutral, horizontal and combined neutral-horizontal types of mandibular growth have a wide face, and children who had vertical and neutral-vertical types of growth have a narrow face.

With regard to the facial tapered angle, which also characterizes the facial shape, it was established that this angle decreases in all groups of teenagers, which is indicative of an elongated narrow face. The biggest difference in the decrease of this parameter is reported in the group with vertical (p=0.001) and neutral-vertical (p>0.001) types of mandibular growth and is on average 6.53º and 5.93º, respectively. In patients with neutral (p=0.001), horizontal (p>0.05) and neutral-horizontal (p=0.001) types of growth, this indicator was closer to the indicator of the control group (average facial shape), but still peculiar to a narrow face. The obtained results are somewhat contrary to the previous parameters and cannot be indisputable in determination of the facial shape in patients with class II_1 anomalies according to Angle.

To assess the face harmony, the gl-sn:sn-me index was used, which is normally equal to 1. In all groups of children, as compared to the control group (1.01±0.03), the index was higher than normal. The highest increase in the index is reported in the groups of patients with neutral (p<0.001), horizontal (p=0.001) and neutral-horizontal (p=0.05) types of mandibular growth (1.15±0.02, 1.19 ±0.02 and 1.14±0.05, respectively). With vertical (p<0.05) and neutral-vertical (p<0.05) type of growth, this indicator is significantly lower if compared to the mentioned groups (1.08±0.05 and 1.05±0.02, respectively), but still higher than normal. Changes in this index indicate a shortened lower part of the face as compared to the average value, which is not harmonious.

Also, the harmony of facial development was determined by the p-p:zy-zy and p-p:go-go indices, which are normally 0.75 and 0.5, respectively.

A decrease in the p-p:zy-zy index (p=0.001) and an increase in the p-p:go-go index were reported in all study groups, regardless of the type of mandibular growth, which indicates that the facial development of patients with occlusion anomalies of class II_1 according to Angle in the late period of mixed bite cannot be considered harmonious.

CONCLUSIONS

A full-face photometric study carried out in 74 patients aged 10-13 years with a distal bite and analysis of the obtained results of its disharmony indicates a characteristic complex of aesthetic symptoms depending on the type of mandibular growth. Vertical and neutral-vertical type of mandibular growth results in a narrow (leptoprosopic) type of face; medium (mesoprosopic) and wide (europrosopic) types of face result from neutral, horizontal and neutral-horizontal types of growth. Irrespective of the type of mandibular growth, all patients with a distal bite have a short lower third of the face. The strongest aesthetic changes are reported in the group of patients with a predominance of the horizontal type of mandibular growth.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

COMPARATIVE ASSESSMENT OF BACTERIAL PERMEABILITY OF A PERSONAL PROTECTIVE RESPIRATORY EQUIPMENT AT DIFFERENT DURATIONS OF ITS CONTINUOUS OPERATION

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ABSTRACT

The aim: To establish the level of antibacterial protection of the studied personal protective respiratory equipment set and its main components and compare antibacterial resistance of the personal protective respiratory equipment set in the presence and absence of filtering components.

Materials and methods: The proposed methodology for assessing biological protection parameters is based on testing the permeability of personal respiratory protection equipment for bacteria by the method of serial dilutions. Also additional culturing of separate components of the protective set on a separate media is carried out. The experiment was also repeated in the absence of filtering elements and when they were replaced by gauze masks.

Results: The use of a fully equipped pneumatic helmet counteracted the penetration of the bacterial aerosol, which was manifested in the absence of growth on the media. The results obtained with the full configuration, as well as the indicators of the spread of bacteria when removing the filter elements and replacing them with gauze masks, showed that the device creates sufficient positive air pressure inside. The latter becomes a restraining factor that does not allow microorganisms to penetrate through the lower circuit.

Conclusions: Increasing the duration of continuous operation of the conceptual model up to 24 hours, increasing the bacterial load on the filters do not lead to a deterioration in the properties of antibacterial protection. Bacterial aerosol did not penetrate into the inner space of pneumatic helmet.

KEY WORDS: pneumatic helmet, laminar air supply, antibacterial protection, Micrococcus luteus

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INTRODUCTION

Prevention of COVID-19 coronavirus infection is an urgent issue of contemporary medicine. The coronavirus pandemic has become an unprecedented event since the last century due to its high incidence, economic loss and expected social consequences [1]. According to the WHO recommendations, the most common measures for coronavirus infection prevention are social distancing, hand and respiratory hygiene. At the same time, the protection of medical staff and persons required to come into contact with the infected patients with COVID-19 coronavirus confirmed is still a topical matter [2].

Personal protective equipment is chosen according to the nature of the interaction with a patient and potential route of infection. In medicine, personal protective equipment is used to protect mucous membranes, respiratory tract, skin and clothes from contact with infections. Suits, gowns, masks, respirators of protection class FFP2, protective goggles, shields and gloves must comply with the state standards [3].

Unfortunately, the use of these means does not provide comprehensive protection against infection. In most cases, these are specialists involved in aerosol-generating procedures: the staff of resuscitation units and departments of morbid anatomy, intensive care units (Fig. 1).

The laboratory staff detecting COVID-19 coronavirus in biomaterials is also a risk group [4]. In addition to the direct threat to medical personnel, there is also a higher probability of infection among students of higher courses of medical institutions [5] and persons with a comorbid combination of somatic pathologies, since the latter more often seek medical help [6-8]. Pneumatic helmet is an innovative system of protection from potentially dangerous infectious agents, which was proposed by scientists of I. Horbachevsky Ternopil national medical university (Ukraine).

The developed concept model of the pneumatic helmet provides positive air flow through a hole in its upper-rear part, where a compressor (powered by a power bank) with an internal and external filter is attached according to the Bayanet principle. It is this part of the model that can most likely turn out to be the place of penetration of infectious agents into the internal space, which makes

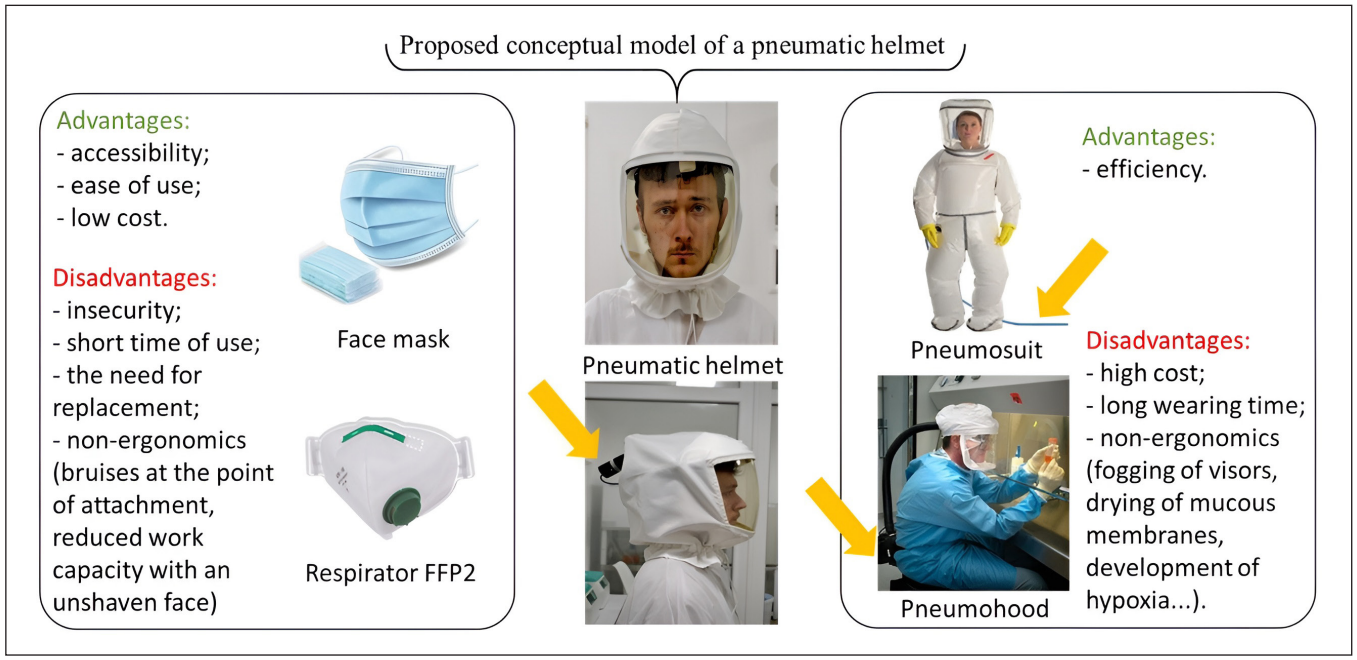

Fig. 1. Comparison of personal protective equipment for respiratory organs

it necessary to check the effectiveness of its biological protection. In order to reduce the cost and simplify the maintenance of the pneumatic helmet, it was decided to remove the exhaust air not through the filter, but through the lower circuit due to the action of positive pressure. This additionally obliges to check the possibility of penetration of microorganisms from the external environment through the lower circuit during the operation of the device under conditions of increased biological load.

THE AIM

The aim of this work was to establish the level of antibacterial protection of the studied personal protective respiratory equipment set and its main components and compare antibacterial resistance of the personal protective respiratory equipment set in the presence and absence of filtering components.

MATERIALS AND METHODS

The proposed methodology for assessing biological protection parameters is based on testing the permeability of personal respiratory protection equipment for bacteria. The suspension of *Micrococcus luteus* is formed with the addition of meat-peptone broth. Its concentration is normalized according to the McFarland turbidity standard of 0,5, which corresponds to $1{,}5 \times 10^8$ colony-forming units per ml (CFU/ml). Further, by dilution, the concentration was reduced to $1{,}5 \times 10^6$ CFU/ml. With a generating device (compressor inhaler) an aerosol is created from this mixture, which is directed to the inlet of the personal protective respiratory equipment (PPRE). The outlet is directed at a Petri dish with meat-peptone broth. The duration of the experiment is 6, 12, 24 hours. After completion, 1 ml of material is cultured on a meatpeptone agar, which is placed in a thermostat for 24 hours at a temperature of 37°C. Evaluation of bacterial contamination is performed by calculating the CFU by multiplying the indicator by the degree of dilution [9, 10]. For counting, the dilution that gave the largest number of colonies in the absence of fusion between them is chosen. Also additional culturing of separate components of the protective set on a separate media is carried out: an external and internal surface of the external filter, compressor, external and internal surface of the internal filter. The study is repeated with a partial configuration of the PPRE – the presence of only external, only internal filter or not using any filter components. A container with a nutrient medium was placed inside a switched-off pneumatic helmet for the indicated period as a control.

The antibacterial protection of the PPRE using a Sheffield's mannequin head is evaluated [11]. The PPRE set is put on the mannequin according to the provided operation rules. The bacterial suspension in the form of an aerosol is fed to the PPRE external filter. After completion, swabbing of the face, neck of the mannequin and the inner surface of the protective glass is performed. The study is repeated with an incomplete set of PPRE. Efficiency is determined by comparison with the results of using the kit without filter components.

The experimental study was conducted during 2021-2022. Testing of the pneumatic helmet concept

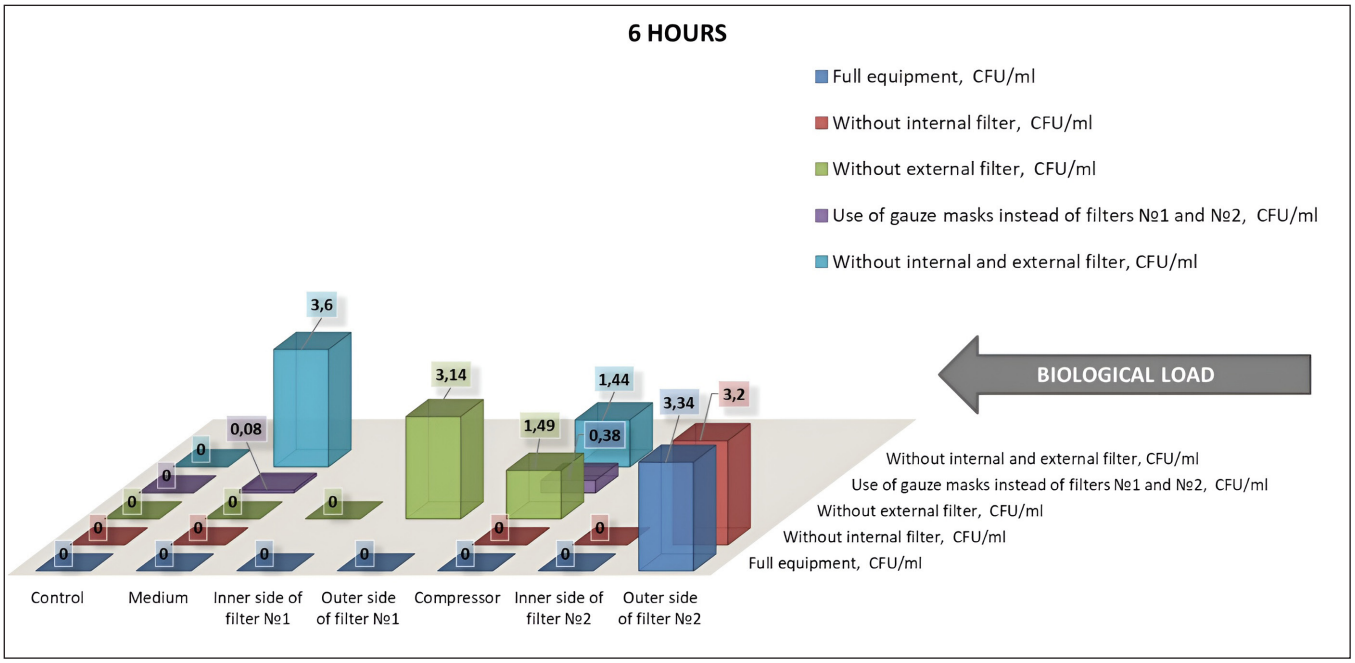

Fig. 2. Evaluation of the effectiveness of the filtering components of the pneumatic helmet for 6 hours of the experiment

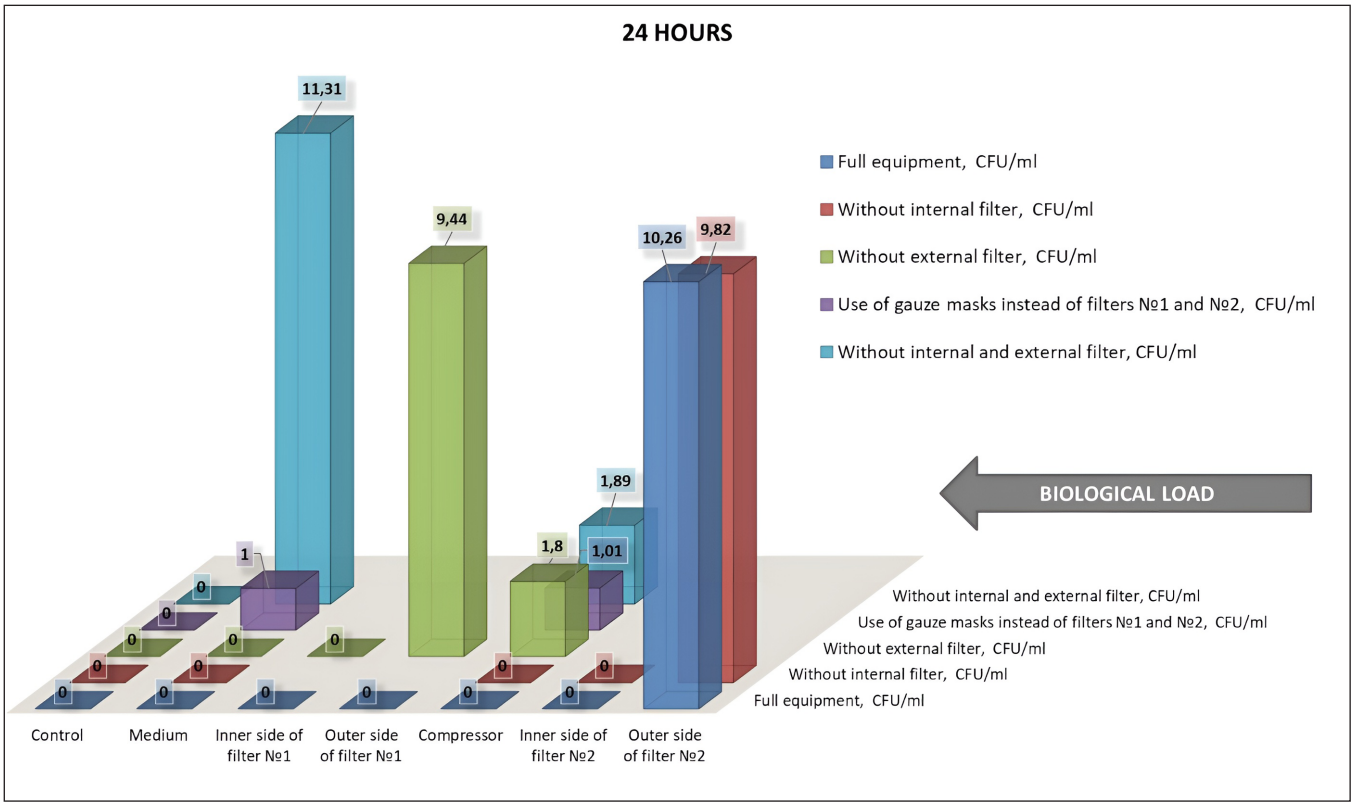

Fig. 3. Evaluation of the effectiveness of the filtering components of the pneumatic helmet for 24 hours of the experiment

model in each of the considered configurations was conducted at least ten times in a series of experiments. The main hypothesis of the research is to confirm the effectiveness of biological protection of the proposed model. The hypothesis is scientific, because it can be denied by revealing the permeability of the system to microorganisms. An additional hypothesis tested in the work is the effectiveness of the proposed biological load system of individual respiratory protective equipment for simulating work in conditions of increased danger. It is possible to deny it in the absence of a statistically significant difference between the results of cultivation after different terms of operation of the pneumatic helmet in various configurations.

Table I. Evaluation of bacterial attachment of the Sheffield's mannequin head for 6 hours of the experiment

Material	Configurations of PPRE		
	Full equipment, CFU/ml	Use of gauze masks instead of filters №1 and №2, CFU/ml	Without internal and external filter, CFU/ml
Swab from the lower part of the face	0	$(0,18\pm0,04)\times10^{2}$*	$(25,70\pm0,47)\times10^{2}$*
Swab from the upper part of the face	0	$(0,55\pm0,07)\times10^{2}$*	$(48,00\pm0,67)\times10^{2}$*
Swab from the neck	0	$(0,10\pm0,03)\times10^{2}$*	$(10,40\pm0,95)\times10^{2}$*
Swab from the inner surface of the protective glass	0	$(0,24\pm0,06)\times10^{2}$*	$(34,50\pm0,50)\times10^{2}$*
Difference of indicators in the group	-	p<0,001	p<0,001

Note: * - a significant difference with full configuration (p<0.001)

Table II. Evaluation of bacterial attachment of the Sheffield's mannequin head for 24 hours of the experiment

Material	Configurations of PPRE		
	Full equipment, CFU/ml	Use of gauze masks instead of filters №1 and №2, CFU/ml	Without internal and external filter, CFU/ml
Swab from the lower part of the face	0	$(6,99\pm0,31)\times10^{2}$*	$(104,60\pm3,90)\times10^{2}$*
Swab from the upper part of the face	0	$(7,23\pm0,25)\times10^{2}$*	$(120,80\pm7,25)\times10^{2}$*
Swab from the neck	0	$(6,68\pm0,24)\times10^{2}$*	$(104,70\pm4,22)\times10^{2}$*
Swab from the inner surface of the protective glass	0	$(6,82\pm0,21)\times10^{2}$*	$(113,10\pm2,75)\times10^{2}$*
Difference of indicators in the group	-	$(6,99\pm0,31)\times10^{2}$*	$(104,60\pm3,90)\times10^{2}$*

Note: * - a significant difference with full configuration (p<0.001)

Statistical processing of the results was carried out using one- and multivariate variance analysis (Statistica 10 software packages). Average sample values of quantitative indicators are given in the form M±m, where M is the arithmetic mean, m is its error. Non-parametric research methods were used due to the non-normal distribution of data found in some groups: Mann-Whitney U-test was used to compare two independent samples, and Friedman test was used to assess dynamic changes within the range. The criterion of reliability was the probability of p, which was equal to or greater than 95,0 % (0,95), that is, the risk of error was less than 5,0 % (p<0,05).

RESULTS

The use of a fully equipped pneumatic helmet counteracted the penetration of the bacterial aerosol, which was manifested in the absence of growth on the media. To compare the effectiveness, the filters of the conceptual model were replaced with gauze masks. The latter were used according to existing recommendations - the mask was replaced every 4 hours of work. At the same time, it was possible to retain 97,8 % of microorganisms, $(0,08\pm0,01)\times10^{4}$ CFU/ml, compared to the results of using a pneumatic helmet without protective barriers – $(3,60\pm0,16)\times10^{4}$ CFU/ml (Fig. 2).

The use of gauze masks instead of filters made it possible to retain 95,1 % of bacteria after 12 hours of work, $(0,35\pm0,01)\times10^{4}$ CFU/ml, compared to the barrier-free version - $(7,08\pm0,30)\times10^{4}$ CFU/ml. When the duration of the experiment was doubled, the number of isolated bacteria from the medium increased by 77,1 % when filters were replaced with gauze masks and by 49,2 % when the device was configured without filter elements. An increase in the concentration of microorganisms in the air due to the extension of the duration of the experiment with full equipment was reflected in the results of seeding from the surface of the external filter - $(6,36\pm0,08)\times10^{4}$ CFU/ml. However, the bacterial aerosol did not penetrate into the inner space of PPRE. Indicators of bacterial attachment to compressor elements differed significantly (p<0,001) when using gauze masks, $(0,71\pm0,02)\times10^{4}$ CFU/ml, and removing filter elements, $(1,93\pm0,07)\times10^{4}$ CFU/ml.

After 24 hours of operation, the concentration of bacteria on the external filter increased by 38,0 % compared to the 12 hours' experiment: $(10,26\pm0,16)\times10^{4}$ CFU/ml and $(6,36\pm0,08)\times10^{4}$ CFU/ml. Replacing the air helmet filters with gauze masks stopped only 91,2 % of the bacterial aerosol, $(1,00\pm0,01)\times10^{4}$ CFU/ml, compared to the experiment with a complete absence of barriers – $(11,31\pm0,43)\times10^{4}$ CFU/ ml (Fig. 3). Growth on the nutrient medium in the complete configuration was not observed.

Parameters of bacterial penetration for the incomplete pneumatic helmet configuration obtained using the mannequin head were statistically different from each other (Table I). It was established that the highest

concentration of microorganisms when replacing filters with gauze masks is observed in the upper part of the face, (0,55±0,07) x10^2 CFU/ml, and the lowest - in the neck area covered by a pneumatic helmet, (0,10±0,03) x10^2 CFU/ml. In the complete absence of filtering elements, these parameters were more pronounced: (48,00±0,67) x10^2 CFU/ml for the upper part of the face and (10,40±0,95) x10^2 CFU/ml for the neck surface. The significance of the difference between them was also high ($p<0,001$). With the full configuration of the device, no signs of growth were detected.

After 12 hours of continuous operation, the presence of microorganisms in the inner space of PPRE was not detected by culture of washings. An increase in the duration of the experiment in the absence of filter elements made it possible to obtain the following indicators of flushing from the area of the mannequin's head (in descending order): (3,34±0,11)x10^2 CFU/ml from the upper part of the face, (3,06±0,07)x10^2 CFU/m from the inner surface of the glass, (2,89±0,11)x10^2 CFU/m from the lower part of the face, (2,38±0,07)x10^2 CFU/m from the neck area covered by a pneumatic helmet. The statistical significance of the difference between these indicators was - $p<0,001$. On average, the indicators increased by 91,3 % compared to the results after 6 hours of work. The attachment of bacteria in the absence of filter elements was more pronounced (in descending order): (70,50±1,43)x10^2 CFU/ml from the upper part of the face, (69,50±1,26)x10^2 CFU/m from the inner surface of the glass, (65,20±2,12) x10^2 CFU/m from the lower part of the face, (59,60±2,07) x10^2 CFU/m from the neck area covered by a pneumatic helmet. At the same time, the statistical difference of the indicated indicators was lower ($p<0,05$). Although the obtained results of bacterial attachment in the absence of filter elements significantly prevail when replacing them with gauze masks, however, in this group, after an additional 6 hours of work, the indicators did not increase so rapidly - 56,4 %.

The growth of bacterial colonies on the medium was not observed after the daily duration of the experiment. The replacement of filters with gauze masks after the expiration of the specified period led to an increase in the rate of bacterial attachment by an average of 60,0 % (Table II). However, no significant difference ($p<0,05$) was found between different areas of the mannequin's head. In the complete absence of filtering elements, an increase in indicators by 40,2 % was noted, which also do not differ statistically.

DISCUSSION

In most cases, testing the effectiveness of filter systems of respiratory protective equipment is carried out with the help of inorganic substances in the composition of gas sprays and aerosols. An example is the study of the effectiveness of filtration of surgical sterilization tissue for the protection of respiratory organs according to its permeability to salt particles, which was also carried out in the conditions of the coronavirus pandemic [12].

The methods of researching indicators of biological protection are not so widespread, which makes it necessary to check their reliability and consider the possibility of involving microbiological laboratories in tests of respiratory protective equipment. When comparing the effectiveness of using sodium chloride particles, polystyrene latex spheres, staphylococci aureus, and bacteriophages for testing the filtration capacity, the more conservative first method was found to have significantly lower efficiency (NaCl). There was also no significant difference between the use of bacteria and bacteriophages, which indicated that the size of the formed aerosol particles is more important for penetration than the size of the microorganisms involved in the experiment. At a concentration of microorganisms equivalent to 1700–2700 CFU per aerosol particle with a diameter of (3,0±0,3) μm, according to this study, the efficiency of the system can be studied in the range of 1-99.9 %. When the concentration of microorganisms increases, the reliability of the determination increases [13].

A significant correlation between the results of using poly-disperse particles and bacteria when testing the filtration capacity of respiratory masks in another study indicates the appropriateness of using both methods for the specified purpose. The difference in electric charges (which are weakly positive for poly-disperse particles and weakly negative for bacteria) did not significantly affect their permeability [14]. The above proves the legality of the obtained results of biological protection for evaluating the effectiveness of the proposed concept model of the pneumatic helmet.

In the course of our research after 6 hours of work the obtained level of antibacterial protection when using gauze masks meets the existing requirements for publicly available means of respiratory protection (more than 95,0 %) [15, 16]. However, the full configuration of the proposed conceptual model was marked by a higher level of biological safety, which is especially important in conditions of increased risk.

Approaching the level of protection of gauze masks to the accepted minimum after 12 hours of work calls into question their effectiveness in conditions of prolonged use or increased bacterial load [17, 18]. Deterioration of barrier capacity may also be associated with the need to regularly replace masks, which is an additional contamination factor [19, 20].

The decrease in the protective effect of a set of masks below the accepted norm of 95 % after 24 hours of work indicates their inability to counteract the increased bacterial load in conditions of long-term operation, in contrast to the filters of the proposed conceptual model.

At the stage of development of the proposed conceptual model, its lower contour was considered as a potential gate for the penetration of microorganisms from the external environment. The results obtained with the full configuration, as well as the indicators of the spread of bacteria when removing the filter elements and replacing them with gauze masks, showed that the device creates sufficient positive air pressure inside. The latter becomes a restraining factor that does not allow microorganisms to penetrate through the lower circuit, thereby simplifying the operation and maintenance of the system.

The results of using the dummy head also show the effectiveness of the proposed pneumatic helmet model in preventing the penetration of bacteria during 6, 12 hours of continuous operation. With an incomplete configuration, the presence of microorganisms on the lower part of the face and neck was statistically ($p<0,05$) lower than similar indicators from other areas.

An increased concentration of bacteria in the air leads to a balancing of the attachment indicators of the surfaces of the inner space of the pneumatic helmet during daily use of PPRE filterless configuration. The faster growth of bacterial penetration indicators when using gauze masks instead of filter elements indicates the ineffectiveness of these means to protect the respiratory organs during prolonged stay in conditions of increased bacterial load. Despite this, a complete configuration of PPRE provides reliable antibacterial protection for 24 hours.

CONCLUSIONS

Extending the duration of the experiment led to an increase in the bacterial load on the surface of the external filter: $(3,34\pm0,08)\times10^4$ CFU/ml for 6 hours, $(6,36\pm0,08)\times10^4$ CFU/ml for 12 hours and $(10,26\pm0,16)\times10^4$ CFU/ml for 24 hours of continuous operation. Despite this, no signs of penetration of microorganisms through the filters and into the inner space of the pneumatic helmet were found. The use of a partial set (without external or internal filters) also did not lead to the isolation of bacteria from the sterile nutrient medium placed in the environment of the model under study, which indicates the presence of a margin of reliability. Instead, when replacing filters with gauze bandages, an increase in the level of bacteriological contamination was observed: $(0,08\pm0,01)\times10^4$ CFU/ml 6 hours, $(0,35\pm0,01)\times10^4$ CFU/ml for 12 hours and $(1,00\pm0,01)\times10^4$ CFU/ml for 24 hours. A significant difference ($p>0,05$) between the specified indicators indicates the possibility of using indicators of biological protection to evaluate the effectiveness of personal protective equipment for respiratory organs. The lack of contamination of the inner space of the pneumatic helmet when it is fully equipped, the lowest bacterial load in the collar area in the absence of the main filtering elements ($(104,70\pm4,22)\times10^2$ CFU/ml for 24 hours) indicate that that the system creates sufficient positive pressure to remove exhaust air through the lower circuit without risk of infection.

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ORIGINAL ARTICLE

EVALUATION OF POSTURAL BALANCE INDICATORS IN HEALTHY INDIVIDUALS

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ABSTRACT

The aim: To determine and generalize the indicators of stabilometry in healthy individuals for their further use as a control group in studies of pathologies of the musculoskeletal system.

Materials and methods: The study was conducted on a stable platform with biofeedback TYMO (Tyromotion). 30 male and female patients aged 18-25 years participated in the study. The following indicators were studied : distance traveled, medial-lateral deviation, anterior-posterior deviation, area of the statokinesiogram (COF), average speed, feedback system, Romberg index. Stabilometry was performed in a bipodal position, standing, in four functional positions: on a hard surface with eyes open and closed, on a soft surface with eyes open and closed.

Results: The reference values of the stabilometric parameters: the traveled distance, medio-lateral deviation, anterior-posterior deviation, the area of the statokinesiogram, the average speed, the feedback system, the Romberg index in healthy individuals aged 18-25 years were determined . When evaluating the feedback system, it was established that the visual component was 34% (32.0; 36.0), the vestibular 34% (32.0; 35.0), the somatosensory 33% (30.0; 36.0). The reflex-driven index was 0.55 (0.46, 0.62), the central nervous system (CNS)-driven index was 1.55 (1.25, 1.89) . The Romberg index M1/M2 was 0.94 (0.78, 1.07), M2/M3 was 0.98 (0.86, 1.10).

Conclusions: The obtained indicators of movement in the sagittal plane, the area of the statokinesiogram, the average speed of movement, the feedback system (visual, vestibular, proprioceptive (somatosensory) components), the Romberg index (RI) can be considered reference values for healthy individuals aged 18-25 years .

KEY WORDS: postural balance, stabilometry, balance test, stability platform, healthy individuals, control group

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INTRODUCTION

The musculoskeletal system is an extremely complex complex consisting of more than 600 muscles, 200 bones, and several hundred tendons. It ensures the implementation of maintaining the posture and movements of a person. The concept of "Posture", "Postural balance" is defined as the ability to maintain and control the general center of mass of the body to maintain balance in static and dynamic positions. Pose is the integration of biomechanical, neurophysiological and neuropsychological factors that interact and mutually compensate at a specific moment in time [1]. To assess the position of the body, the central nervous system receives information for analysis and subsequent reaction due to three main components: visual, vestibular and proprioceptive [2, 3], which affect the regulation of muscle activity at different levels of the CNS: spinal, trunk, cortical, which organize their activities based on the processing of afferent information [4]. Postural stabilization is performed by muscles, the function of which is to maintain the vertical position of the body and overcome the force of gravity. They have the functional ability to contract over a long period of time, maintaining the posture. But this property often leads to an overload of postural muscles, a violation of their trophism, especially during long-term static loading [5].

The somatosensory system receives information about the relative location of body parts in a static position (so-called proprioception) and in dynamic positions. The system receives this information from peripheral sources, muscles, joint capsules and soft tissue receptors (muscle spindles, Ruffini endings). This system plays an important role in posture regulation [6].

Postural stability is the ability to maintain the vertical position of the body within the boundaries of the support area [7]. Postural stability (including a certain

rigidity of the human body under gravity) is provided by postural reflexes, which are of two types:
– postototonic reflexes, which limit (if necessary) the number of degrees of freedom of joints due to fixation by tonic muscles. Thus, the spine is limited in mobility by the paravertebral muscles; mobility in the hip, knee and ankle joints and the atlanto-occipital articulation is limited by certain muscles.
– positioning reflexes: upon deviation from the vertical, receptors of the vestibular apparatus and proprioceptors are activated, which quickly provide information to restore body position and balance.

The vestibular system is mainly involved in slow and high-amplitude movements, which are absent in the normal upright posture of a healthy person [8].

The main reliable method for assessing postural balance is stabilometry, which allows detecting changes in the static-dynamic function, the degree of their compensation, the characteristics of movement disorders, the severity of the impact on posture maintenance of visual, vestibular and proprioceptive components, the presence of sensory conflict - a condition in which there is a mismatch of degrees the influence of sensory information from elements of the feedback system [9-11].

A special advantage of this method is the possibility of digital expression of all parameters and their accounting, which makes it possible not only to assess the severity of changes in balance and the direction of therapeutic influence, but also to document the effectiveness of one or another method of treatment clearly [12].

Despite a sufficiently large number of studies, there is still no consensus on the interpretation of stabilometry indicators due to the fact that the studies were conducted using different equipment, with different designs, and there is no agreement among researchers on the concept of "norms" for different age groups and patients with different pathologies or healthy persons [13-18].

In Ukraine, such studies are generally episodic and do not reflect the state of the problem [19, 20].

THE AIM

The aim is to determine and generalize the indicators of stabilometry in healthy individuals for their further use as a control group in studies of pathologies of the musculoskeletal system.

MATERIALS AND METHODS

The study was conducted on a stable platform with biological feedback TYMO (Tyromotion) with the assistance of the REHAB project of the Erasmus+ program on the basis of Ternopil National University named after I.Ya. Gorbachevskiy Ministry of Health of Ukraine and the center of kinesiology and therapeutic massage "Milon". 30 patients, i.e.15 women and 15 men, took part in the study.

The criteria for inclusion in the study were the following ones:
- Young men and women aged 18-25
- Absence of acute and chronic diseases in the anamnesis and at the time of examination
- Absence of injuries, operations and diseases of the musculoskeletal system
- Absence of vestibular disorders
- Absence of posture disorders
- Satisfactory physical development
- Persons who do not engage in sports professionally.

Indicators that were evaluated in the examinees: traveled distance, medial-lateral deviation, anterior-posterior deviation, area of the statokinesiogram (COF), average speed, load distribution, feedback system, Romberg index. Stabilometry was performed in a bipodal position, standing, in four functional positions: on a hard surface with eyes open and closed (M1 and M2), on a soft surface with eyes open and closed (M3 and M4).

During the study, the patient was in the main position without additional fixation points, the effect of extraneous sound, light, cognitive stimuli is excluded. For the objectivity of the examination results, the length of the foot, weight, height, and age of the patient are taken into account in the work of the program.

Statistical processing of measurement results was carried out using STATISTICA 10.0 software. The check for the normality of the distribution law was carried out using the Shapiro-Wilk test, the normal-probability graph, the coefficients of asymmetry and kurtosis. According to the results of the inspection, non-compliance with the normal law of the distribution of the studied indicators was established and, as a result, the use of non-parametric methods in the comparative analysis of the obtained data and their presentation in the form of the median and interquartile range - Me (Lq; Uq).

All patients were informed about the aim of the clinical study and gave written informed consent for their participation in it. Confidentiality of information about the patient's identity and state of health was preserved.

RESULTS

For the convenience of discussion of the results, a description of the following indicators, which are presented in Table I, should be entered:
– M1 – standing position on a hard surface with open eyes,
– M2 – standing position on a hard surface with closed eyes,

Table I. Indicators of distances, area of the statokinesiogram and frequency according to the results of the balance test

№№	Indicator	Examination conditions			
		M1	M2	M3	M4
1	Distance traveled	22.25 (20.0; 25.25)	30.0 (28.0; 34.0)	39.5 (37.0; 46.5)	77.0 (69.0; 95.0)
2	Medial-lateral deviation	1.0 (1.0; 2.0)	1.0 (1.0; 2.0)	2.0 (1.0; 3.0)	3.0 (2.0 ;4.0)
3	Front-back deviation	1.0 (1.0; 2.0)	2.5 (2.0; 3.0)	3.0 (2.0; 4.0)	4.0 (3.0; 6.0)
4	COF area	0.2 (0.1; 0.3)	0.25 (0.2; 0.3)	0.5 (0.3; 0.55)	1.3 (1.2; 2.0)
5	Average speed	5.0 (4.5; 6.0)	4.0 (4.0; 5.0)	5.0 (4.0; 5.0)	4.0 (4.0; 5.0)
6	Frequency analysis	0.86 (0.74, 0.93)	0.87 (0.80, 0.92)	0.84 (0.80, 0.89)	0.89 (0.80, 0.93)

- M3 – standing position on a soft surface with open eyes (when standing on a soft surface, the impulse from pressure mechanoreceptors on the plantar surface of the foot and joint receptors decreases, but does not affect the work of muscle receptors and is one of the methods of differentiating the central and peripheral dysfunction),
- M4 – standing position on a soft surface with closed eyes,
- distance traveled: the distance the body moves to maintain balance in centimeters,
- mediolateral deviation: movement in the frontal plane in centimeters,
- anterior-posterior deviation: movement in the sagittal plane in centimeters,
- COF area is the area of the statokinesiogram when maintaining balance (cm 2),
- average movement speed: the average value of body movement speed during the balance test (cm/s),
- feedback system: visual, vestibular, proprioceptive (somatosensory) components in percentages (the feedback system is taken as 100%, so each of its components is also presented as a percentage),
- the value of the Romberg index (RI) provides information about the dependence of the movement strategy on the visual channel of receiving information. The components of this indicator refer to frequency indicators. The Romberg index demonstrates the relationship between the visual analyzer, lateral deviation and vertical elevation of the foot. The Romberg index allows you to determine the participation of the proper proprioceptor component in maintaining the vertical position. It should be noted that during physiological posture regulation, the magnitude of body oscillations is lower than the threshold perceived by the vestibular apparatus. An important circumstance is also taking into account the placement of the feet on the stable platform and the length of the examinee's foot, because it is clear that the size of the so-called "clinical base" - the distance between the balance axes of the feet in the main position and the size of the foot affect the placement of the center of mass and maintenance of balance. For the objectivity of the examination results, all these indicators, as well as the patient's weight, height, age, are provided and taken into account in the work of the program.
- the reflex-controlled index assesses the presence of reflex sequences of movements,
- controlled by the central nervous system (CNS) index assesses the presence of CNS-generated movement sequences that are controlled by the brain.

When evaluating the feedback system, it was established that the visual component was 34 (32.0; 36.0), the vestibular component was 34 (32.0; 35.0), and the somatosensory component was 33 (30.0; 36.0). It is believed that theoretically each of these components should be equal to 33%, which was noted in the group of our examinees. The reflex-controlled index was 0.55 (0.46, 0.62), the CNS-controlled index was 1.55 (1.25, 1.89). The reflex-controlled index is considered satisfactory at values close to 0.5 and prognostically unsatisfactory at values close to 1.5. The value of the managed central nervous system index: good with an indicator of about 1.5 and unsatisfactory - about 0.5. The Romberg index M1/M2 was 0.94 (0.78, 1.07), M2/M3 was 0.98 (0.86, 1.10). The Romberg index value greater than 1.0 indicates a greater volume of postural deviation at the stage with closed eyes, but no deviations were found in the results of our study.

DISCUSSION

Standardization of stabilometry was first proposed at the 1983 Posturography meeting in Kyoto, but has not been adopted worldwide, and each country continues to use unique regional measurement methods [21]. In 2013, the International Committee for Standardization of Clinical Stabilometry of the International Society for Posture and Gait Research (ISPGR) proposed metrological indicators of posturographic instruments [3]. Evaluation of reference values of stabilometric indicators in healthy individuals was carried out in a relatively small number of studies or with a small number of subjects [9, 22-25]. Only a few sources evaluated a significant number of patients of different age groups [13, 26].

However, it is clear that certain normative indicators are important for the clinical assessment of balance disorders. A comparison between the data of patients and the data of healthy people can reveal the presence of hidden balance disorders and apply intervention strategies. Normative results are necessary to quantify the changes that occur during the rehabilitation period, and can serve as a criterion for evaluating the effectiveness of interventions.

The Onofrei R.R. study of healthy young adults indicates that physical activity levels affect balance, but no gender differences were found in balance scores in healthy young adults. [27]. Another study [28] reported that posturography mean values for stability limit, ellipse area, and sway rate for stimuli with visual-vestibular interaction showed significant gender differences, and in all cases women scored lower than men Goble DJ [13] states that there is no relationship between height and weight and postural sway, but Emara A. [29] states that an increase in body mass index affects various sensory systems needed to maintain balance control and the used motor strategy, and older adults with obesity had an increased reliance on the vestibular system to maintain balance.

The question of the impact of various types of physical activity on the balance function remains debatable, in particular [30], claim that postural control is improved by balance exercises and conversely, strength or multicomponent exercises did not reliably affect balance performance. On the contrary, a study [31] notes that active strength training can improve attention control of balance. Several studies have found that the ability of the three sensory systems involved in dynamic postural balance is equally impaired after both anaerobic and aerobic exercise protocols [32-35] therefore we examined patients in the morning without exercise and exercise before the study.

It should be noted that some authors note that the data of registration of postural balance indicators on different devices may not always correlate with each other [36], so the results obtained in our study can be considered normative for the stable platform with biological feedback TYMO.

We believe that when conducting preventive examinations of patients, it is worth using the stabilometry method to detect initial dysfunctions and prevent the development of diseases of the musculoskeletal system.

CONCLUSIONS

The obtained indicators of movement in the sagittal plane, the area of the statokinesiogram, the average speed of movement, the feedback system (visual, vestibular, proprioceptive (somatosensory) components), the Romberg index can be considered reference values for healthy individuals aged 18-25 years .

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ORIGINAL ARTICLE

DIFFERENTIAL DRUG CORRECTION CYTOKINE AND PROSTAGLANDIN CONTENT IN BLOOD AND GINGIVAL FLUID IN GENERALIZED PERIODONTITIS AGAINST THE BACKGROUND OF DIFFERENT RESPONSIVENESS OF THE ORGANIZATION

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ABSTRACT

The aim is to study the dynamics of prostaglandins and cytokines in the blood and gingival fluid against the background of differential drug correction in patients with generalized periodontitis with different body reactivity.

Materials and methods: 216 people aged 45 between 55 years with a diagnosis of generalized periodontitis of II, III degree of severity, chronic course were examined. Depending on the state of reactivity of the organism, the patients were divided into three groups: the first one consisted of people with normoreaction; the second group included patients with hyperreaction; the third group was made up of people with hyporeaction. The patients underwent patch surgery after the initial therapy. Initially, on the 1st, 2nd, 4th, 6th and 9th day after the operation, the content of prostaglandins (PG) E, E2, F2alpha and cytokines (IL-1β, IL-6, TNF, IL-4) in the blood and gingival fluid was performed. Patients with impaired body reactivity were treated with the proposed differential drug correction of cytokines and prostaglandins. Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0.

Results: The proposed differential drug correction in patients with generalized periodontitis against the background of hyper- and hyporeactivity of the body brings the content of IL-1β, IL-6, TNF, IL-4 to that of normal body reactivity, which ultimately restores the disturbed balance of pro- and anti-inflammatory cytokines in the blood and gingival fluid. On the 9th day, the content of all proinflammatory cytokines in the main groups was normalized and was commensurate with that of the body's normal response ($p>0.05$). Differential drug correction led to normalization of the prostaglandin balance index on day 9 after flap surgery ($p>0.05$), indicating the establishment of a normal balance of eicosanoids in the blood and approximating the values of pro- and anti-inflammatory fractions of prostaglandins to those of normal body reactivity.

Conclusion: Correction of altered parameters in patients with generalized periodontitis accompanied by impaired (hyper- and hypo-) reactivity of the body with bringing them to values that are typical for normoreactivity is considered to be a condition for optimizing mucosal wound healing after surgery and further stabilization of periodontal tissues.

KEY WORDS: periodontitis, reactivity of the organism, drug treatment, cytokines and prostaglandins

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INTRODUCTION

General and local drug treatment of generalized periodontitis has an etiologic, pathogenetic and symptomatic focus [1-3]. Pathogenetic drug correction is aimed at relieving the leading links in the development of the disease [4, 5]. The prescription of nonsteroidal anti-inflammatory drugs (NSAIDs) (mainly selective COX-2 inhibitors to reduce prostaglandin E2 levels) is justified. There is experience in the use of 3% acetylsalicylic ointment, 3% orthophene ointment, 1% Voltaren emulsion (Switzerland) as part of periodontal dressings, diclofenac sodium medicated paste, flubiprofen gel, mouthwash with 0.1% ketorolac solution and 1.5% dexibuprofen solution, toothpaste containing 1% and 3% ketoprofen. Selective COX-2 inhibitors have been used in the complex treatment of generalized periodontitis. These are drugs such as etoricoxib, rofecoxib, celecoxib, meloxicam, nimesulide, etodolac, etc. [6]. Schemes of local (immobilization on the sorbent «Enterosgel») and general use of a selective COX-2 inhibitor - meloxicam (Melbek), Turkey, have been proposed [7]. Immunocorrective therapy is used, which stimulates immune processes, specifically activates immunocompetent cells (T and B lymphocytes) and additional immune factors (macrophages, secretory immunoglobulins, cytokines, etc.) [8]. High therapeutic

efficacy of immunomodulators has been shown. A special place among immunomodulatory drugs is occupied by phytoadaptogens. Clinical and immunological studies have revealed a pronounced immunomodulatory effect of biotritol-based adaptogen on the parameters of both local and systemic immunity in patients with generalized periodontitis [9]. Cytokine therapy is used, which includes the administration of drugs of specific cytokines or drugs of cytokine antagonists of IL-1, TNF, interferon drugs (laferon, immuneron), interferon inducers (amizon, amixin) [10-13]. Thus, modern approaches to the medical treatment of patients with generalized periodontitis are based on the relief of the leading links in the etiopathogenesis of the disease. The correct orientation of this tactic is confirmed by numerous literature data on the high clinical efficacy of the proposed and implemented drug treatment regimens for generalized periodontitis. However, the issues of clarification of the key mechanisms of development and course of the disease in each specific clinical case and the development of differentiated approaches to the choice of a complex of medications for general and local use, depending on exo- and endoparodontic pathogenic factors that most affect the pathogenesis of generalized periodontitis, remain relevant.

THE AIM

The aim of this study was to investigate the dynamics of prostaglandins and cytokines in blood and gingival fluid against the background of differential drug correction in patients with generalized periodontitis with different body reactivity.

MATERIALS AND METHODS

216 patients (82 men and 134 women), aged 45 between 55 years, with the diagnosis of generalized periodontitis of II, III degree of severity, chronic course were examined. The diagnosis was made on the basis of clinical examination, radiography, determination of periodontal samples in accordance with the International Classification of Diseases ICD-10. Depending on the state of reactivity of the body, the patients were divided into three groups: the first one included patients with normoreaction (132 people, 61%); the second group consisted of patients with hyperreaction (46 people, 21%); the third one contained patients with hyporeaction (38 people, 18%). The division of patients into groups depending on the state of reactivity of the organism was performed on the basis of the identified clinical and laboratory differences. All the patients underwent comprehensive treatment of generalized periodontitis in the amount recommended by the Ministry of Health of Ukraine - Order №566 of 23.11.04. "On approval of the Protocols for the Provision of Medical Care". All the patients with generalized periodontitis of II, III degrees of severity after completion of the complex of initial therapy, according to the indications, underwent patch surgery. Immediately after the surgical intervention, differential medication support was started for patients of the main groups with hyper- (2A) and hypoergic (3A) types of inflammation. Patients with normoergic type of inflammation (comparison group and control groups 2B and 3B) were not additionally offered drug therapy. Since in case of hyperreactivity of the body there is an increased content of proinflammatory prostaglandins E, E2, it is advisable to use an appropriate complex of pathogenetically adequate medications to normalize these parameters. The increased content of proinflammatory prostaglandins determines the validity of the use of nonsteroidal anti-inflammatory drugs (NSAIDs) with the active ingredient nimisulide (UA/9855/01/01 of 17.07.2019; Order 1625(2) of 17.07.2019). The universal mechanism of action of NSAIDs is considered to be the blockade of COX (prostaglandin endoperoxide synthetase) synthesis, a key bifunctional enzyme involved in the regulation of prostaglandin synthesis. The drug containing nimisulide was administered orally in 1 sachet (the sachet contains granules for the preparation of a suspension) 2 times a day (200 mg per day) for 5-7 days. Significant activation of cytokines in case of hyperreaction and reduced levels in case of hyporeaction determine the expediency of using immunocorrective drugs. We opted for the high molecular weight chemically pure immunomodulator Liasten with the active ingredient glucosaminylmuramylpentapeptide (UA/14212/01/01 of 13.05.2020; Order 1128(2) of 13.05.2020). The drug stimulates the production of proinflammatory cytokines only at their initially low levels, and reduces them at initially elevated levels. We used the tablet form of the drug (2 mg) immobilized on a sorbent as a periodontal dressing.

The content of prostaglandins (PG) E, E2, F2alpha and cytokines (IL-1β, IL-6, TNF, IL-4) was determined in the blood serum. The corresponding cytokines were determined in the gingival fluid. In the morning, blood and gingival fluid were collected on an empty stomach. Venous blood was taken from the ulnar vein (5 ml). Gingival fluid was collected with standard paper pins (#25) by immersing them without force into the gingival sulcus or periodontal pockets for 30 seconds in the upper canines. The state of cytokine regulation was determined by enzyme-linked immunosorbent assay (ELISA) using commercial reagent kits ProCon IL-1β, ProCon IL-6, ProCon TNF, ProCon IL-4. The optical density at a given wavelength was measured using a spectrophotometer. The radioimmune method was used to determine GH. The dynamics of the studied laboratory parameters was evaluated on the 1st, 2nd, 4th, 6th and 9th days after surgical treatment.

Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0 (STA862D175437Q).

RESULTS

The results of determining the cytokines IL-1β, IL-6, TNFα , IL-4 in the blood of patients with generalized periodontitis against the background of normal, hyper- and hyporeactivity of the body after surgery are presented in the table (Table I).

As can be seen from this table, in patients with generalized periodontitis of the main groups, the dynamics of the content of these cytokines against the background of the proposed drug support was unidirectional in the direction of increasing their concentration. This dynamics of indicators is similar to that of normal body reactivity. On the 1st day after surgery in groups 2A and 3A, the greatest increase in proinflammatory cytokines was noted ($p<0.05$). The content of these cytokines significantly exceeded the control values on the 2nd day, but to a lesser extent compared to the 1st day. On days 4 and 6, a decrease in the content of all proinflammatory cytokines in the blood serum was recorded. On the 9th day, the content of all proinflammatory cytokines in the main groups was normalized and was commensurate with that in patients with AP with normal body reactivity.

The dynamics of the anti-inflammatory cytokine IL-4 in the blood of patients of groups 2A and 3A differed from that of IL-1β, IL-6, and TNFα . The content of IL-4, gradually increasing from the 1st day of observation, reached a peak on the 6th day (the content, respectively, was 1.8 and 1.7 times higher compared to the baseline, $p < 0.05$). It should be noted that during this observation period, the values of the indicator differed significantly from those in the control and were, respectively, 2.1

Table I. Cytokine content of IL-1β, IL-6, TNFα , IL-4, IRC in the blood of patients with generalized periodontitis in normal, hyper- and hyporeactivity after surgical treatment (M±SE)

Indicators.	Terms. observation	Patient groups		
		norm-reaction (n = 132)	overreaction (n = 23)	Hyporesponse (n = 19)
IL-1β, pkg/mL	initially	73,6 ± 16,4	74,2 ± 28,0	71,8 ± 28,8
	1st day	142,1 ± 20,8 *	165,0 ± 32,1 *	115,8 ± 30,0
	2nd day	130,6 ± 18,2 *	148,2 ± 26,0 *	110,0 ± 30,5
	4th day	125,9 ± 18,0 *	134,4 ± 25,8	106,9 ± 28,5
	6th day	94,5 ± 17,2	103,5 ± 25,4	96,6 ± 25,4
	9th day	74,2 ± 16,0	88,8 ± 24,4	77,8 ± 25,0
IL-6, pkg/mL	initially	86,1 ± 14,4	87,0 ± 32,4	85,2 ± 33,5
	1st day	126,4 ± 18,0 *	130,9 ± 34,9	119,7 ± 35,0
	2nd day	115,2 ± 17,6 *	123,6 ± 32,6	117,0 ± 34,3
	4th day	104,1 ± 16,8	112,8 ± 30,0	105,8 ± 36,0
	6th day	96,5 ± 15,0	108,2 ± 30,2	97,1 ± 34,8
	9th day	84,9 ± 14,2	99,0 ± 29,7	85,0 ± 30,2
FNPα , pkg/mL	initially	75,9 ± 12,8	76,2 ± 34,4	74,5 ± 35,7
	1st day	111,8 ± 17,4 *	136,1 ± 34,4	128,0 ± 34,2
	2nd day	106,4 ± 16,7 *	130,4 ± 34,0	108,6 ± 34,0
	4th day	104,8 ± 16,5	122,1 ± 30,4	118,2 ± 30,3
	6th day	82,6 ± 13,4	97,0 ± 28,2	86,9 ± 28,9
	9th day	77,1 ± 12,6	82,9 ± 28,0	79,8 ± 28,5
IL-4, pkg/ml	initially	42,1 ± 8,4	43,8 ± 15,8	41,6 ± 18,8
	1st day	50,6 ± 8,8	62,8 ± 22,0	56,7 ± 18,8
	2nd day	54,4 ± 9,2	58,8 ± 16,9	55,1 ± 18,0
	4th day	68,9 ± 9,7 *	69,5 ± 17,8	64,5 ± 19,4
	6th day	74,5 ± 10,0 *	77,5 ± 16,5 *	70,6 ± 19,1 "
	9th day	48,2 ± 8,0	53,2 ± 15,6	54,0 ± 16,0
CPI	initially	1,06 ± 0,04	1,08 ± 0,13	1,09 ± 0,15
	1st day	2,34 ± 0,07 *	2,38 ± 0,20 *	2,37 ± 0,19 *
	2nd day	2,01 ± 0,07 *	2,14 ± 0,26 *	2,02 ± 0,17 *
	4th day	1,68 ± 0,06 *	1,69 ± 0,19 *	1,73 ± 0,16 *
	6th day	1,22 ± 0,05 *	1,38 ± 0,18	1,28 ± 0,15
	9th day	1,16 ± 0,05	1,24 ± 0,16	1,23 ± 0,15

Note: * - $p < 0.05$ vs. initial values

"- $p < 0.05$ vs. values at normal body reactivity

Table II. The content of cytokines IL-1β, IL-6, TNFα , IL-4 in the gingival fluid of patients with generalized periodontitis in normal, hyper- and hyporeactivity after surgical treatment (M±SE)

Indicators.	Terms. observation	Patient groups		
		norm-reaction (n = 132)	overreaction (n = 23)	Hyporesponse (n = 19)
IL-1β, pkg/mL	initially	27,3 ± 1,4	28,2 ± 2,4	26,9 ± 2,4
	1st day	68,1 ± 2,8 *	76,8 ± 4,4 *	62,0 ± 4,6 *
	2nd day	59,0 ± 1,8 *	62,0 ± 4,2 *	58,2 ± 4,1 *
	Day 4	52,9 ± 1,9 *	54,4 ± 3,8 *	49,8 ± 3,8 *
	6th day	34,5 ± 1,5 *	38,5 ± 2,9 *	32,7 ± 2,8 *
	9th day	28,2 ± 1,4	26,8 ± 2,7	25,9 ± 2,70
IL-6, pkg/mL	initially	123,6 ± 5,4	124,2 ± 9,7	122,8 ± 9,6
	1st day	264,4 ± 8,0 *	271,2 ± 10,2 *	244,8 11,0 *
	2nd day	189,5 ± 7,3 *	194,5 ± 9,8 *	168,5 10,8 *
	Day 4	134,1 ± 6,1	139,6 ± 8,8	131,2 ± 9,8
	6th day	128,6 ± 5,0	132,0 ± 8,5	123,8 ± 9,6
	9th day	124,9 ± 4,8	126,4 ± 8,4	122,4 ± 8,6
FNPα , pkg/mL	initially	45,4 ± 4,8	46,1 ± 9,0	44,8 ± 8,8
	1st day	116,2 ± 7,1 *	126,7 ± 10,5 *	108,8 ±10,0 *
	2nd day	101,0 ± 6,7 *	107,9 ± 9,2 *	96,5 ± 9,2 *
	Day 4	85,9 ± 5,1 *	92,0 ± 9,0 *	81,9 ± 9,0 *
	6th day	62,7 ± 4,4	56,8 ± 8,9	56,9 ± 8,7
	9th day	47,3 ± 4,2	49,4 ± 8,5	45,8 ± 8,5
IL-4, pkg/mL	initially	16,9 ± 2,4	17,2 ± 5,1	16,7 ± 5,3
	1st day	21,8 ± 2,8	27,7 ± 6,1	20,2 ± 6,2
	2nd day	24,9 ± 2,2	26,8 ± 6,0	22,8 ± 6,9
	Day 4	48,1 ± 3,7 *	52,2 ± 6,4 *	44,7 ± 8,2 *
	6th day	54,5 ± 3,8 *	57,8 ± 6,2 *	52,8 ± 6,1 *
	9th day	18,1 ± 2,2	19,6 ± 5,6	17,6 ± 5,8

Note: * - $p < 0.05$ vs. initial values

" - $p < 0.05$ vs. values at normal body reactivity

and 2.3 times lower ($p < 0.05$). On day 9, the value of IL-4 in the main groups remained higher than the initial value and became commensurate with that in the comparison group. As can be seen from this table, the dynamics of the cytokine balance index in the main groups coincided with that of the normoreactivity of the body and was monophasic with a peak on the 1st day. The increase in the balance index on the 1st day after surgery against the background of the proposed medical support testifies in favor of a shift in balance towards the predominant activation of proinflammatory cytokines. Starting from day 2, in groups 2A and 3A, as well as in the comparison group, the equilibrium began to level off - the index decreased with a minimum value by the end of the observation. At the same time, the IRC was, respectively, 1.24 ± 0.16 and 1.23 ± 0.15, which did not differ significantly from the initial values and those of the normal body reactivity ($p > 0.05$). Thus, the proposed targeted drug correction in patients with generalized periodontitis against the background of hyper- and hyporeactivity of the body approximates the values of cytokines to those of normal body reactivity, which ultimately restores the disturbed balance of pro- and anti-inflammatory cytokines in the blood.

The results of determining cytokines (IL-1β, IL-6, TNFα, IL-4) in the gingival fluid of patients with generalized periodontitis in normal, hyper- and hyporeactivity after surgery are presented in the table (Table II).

As can be seen from this table, the dynamics of the content of these cytokines in the gingival fluid of patients with generalized periodontitis of the main groups was similar to that in the blood serum and was characterized by their increase with maximum values of proinflammatory cytokines on day 1, anti-inflammatory cytokines - on day 6 and subsequent normalization of indicators by the end of the observation. The differences concerned the amplitude of changes in these parameters: in the gingival fluid, the changes were significantly more pronounced compared to those in the blood at all follow-up periods ($p < 0.05$). On the 1st day after surgery, the greatest increase in proinflammatory cytokines was noted in groups 2A and 3A ($p < 0.05$). The content of these cytokines significantly exceeded the control values on day 2, but to a lesser extent compared to day 1. On days 4 and 6, a decrease in the content of IL-1β, IL-6, and TNFα in the gingival fluid was recorded. On the 9th day, the content of all proinflammatory cytokines in the main groups was normalized and was commensurate with that in patients with HP with normal body reactivity.

Table III. The content of prostaglandins F2α and E2, the ratio of PG F2α / PG E2, the value of the prostaglandin balance index (PBI) in the blood of patients with generalized periodontitis in normal, hyper and hyporeactivity of the body after surgical treatment (M±SE)

Indicators.	Terms. observation	Patient groups		
		Normoreaction (n = 132)	overreaction (n = 23)	Hyporesponse (n = 19)
GHG F2α, ng/ml	Initially	0,534 ± 0,051	0,553 ± 0,153	0,509 ± 0,149
	1st day	0,986 ± 0,062 *	1,101 ± 0,160 *	0,912 ± 0,157 *
	2nd day	1,062 ± 0,063 *	1,079 ± 0,168 *	1,039 ± 0,159 *
	4th day	0,890 ± 0,054 *	0,920 ± 0,152 *	0,886 ± 0,150 *
	6th day	0,740 ± 0,055 *	0,778 ± 0,145	0,725 ± 0,148
	9th day	0,682 ± 0,050 *	0,695 ± 0,140 "	0,677 ± 0,144
GHG E2, ng/ml	Initially	2,296 ± 0,330	2,352 ± 0,538	2,090 ± 0,536
	1st day	2,532 ± 0,322	2,750 ± 0,560	2,485 ± 0,608
	2nd day	2,314 ± 0,282	2,462 ± 0,558	2,270 ± 0,600
	4th day	2,453 ± 0,290	2,460 ± 0,558	2,384 ± 0,614
	6th day	3,006 ± 0,340 *	3,208 ± 0,600 *	2,885 ± 0,626 *
	9th day	2,764 ± 0,307	2,785 ± 0,582	2,687 ± 0,577
GHG F2α / GHG E2	Initially	0,25 ± 0,02	0,23 ± 0,06	0,24 ± 0,05
	1st day	0,40 ± 0,03 *	0,40 ± 0,06 *	0,36 ± 0,06 *
	2nd day	0,46 ± 0,03 *	0,44 ± 0,06 *	0,45 ± 0,08 *
	4th day	0,38 ± 0,02 *	0,37 ± 0,05 *	0,37 ± 0,07 *
	6th day	0,25 ± 0,02	0,24 ± 0,04	0,25 ± 0,06
	9th day	0,25 ± 0,01	0,25 ± 0,05	0,25 ± 0,06
IRPG	Initially	1,26 ± 0,09	1,22 ± 0,15	1,23 ± 0,16
	1st day	2,03 ± 0,10 *	1,82 ± 0,17 *	1,86 ± 0,17 *
	2nd day	2,46 ± 0,11 *	2,32 ± 0,19 *	2,35 ± 0,19 *
	4th day	1,86 ± 0,09 *	2,01 ± 0,19 *	2,04 ± 0,18 *
	6th day	1,32 ± 0,08	1,25 ± 0,14	1,26 ± 0,16
	9th day	1,00 ± 0,05 *	1,04 ± 0,14	1,03 ± 0,16

Note: * - $p < 0.05$ vs. initial values

"- $p < 0.05$ vs. values at normal body reactivity

The dynamics of the anti-inflammatory cytokine IL-4 in the gingival fluid of patients of groups 2A and 3A differed from that of IL-1β, IL-6, TNFα - the peak of IL-4 content was noted on day 6. This indicator had the same dynamics in the case of normal body reactivity. On the 9th day, the value of IL-4 in the main groups remained higher than the initial value and was commensurate with that in the comparison group. Thus, the proposed targeted drug correction in patients with generalized periodontitis against the background of hyper- and hyporeactivity of the body approximates the values of cytokines in the gingival fluid to those of normal body reactivity, which ultimately restores the disturbed balance of pro- and anti-inflammatory cytokines.

The results of determining the content of different fractions of prostaglandins in the blood of patients with generalized periodontitis against the background of normal, hyper- and hyporeactivity of the body after surgery are presented in the table (Table III).

As can be seen from this table, initially (before surgery), the concentration of anti-inflammatory PG F2α in the blood of patients with generalized periodontitis with targeted drug correction differed from that in the first group ($p > 0.05$). Surgical intervention led to an increase in the content of this eicosanoid in the main groups and the comparison group with monophasic dynamics. The maximum value occurred on day 2 ($p < 0.05$) with a subsequent decrease until the end of the study. The level of proinflammatory PG E2 in the blood of patients with generalized periodontitis was also initially slightly different from that in the first group ($p > 0.05$). Surgical intervention caused a similar initial increase in the concentration of PG E2 in the main groups (2A and 3A) and the comparison group ($p > 0.05$). However, both the amplitude of changes and the dynamics of the index differed from that of PG F2α: the changes were less pronounced, the dynamics were biphasic with maximum values on days 1 and 6. On the 2nd day, a slight decrease in the concentration of GH E2 was observed - at this point, the different direction of changes in the content of GH F2α and GH E2 is noteworthy. The former reached maximum peak values, while the latter, on the contrary, was minimal. Subsequently, the second peak in the concentration of GHG E2 was observed in the main groups, which was more pronounced than the first. Its values, on average, were 1.4 times higher than the initial ones ($p<0.05$). A similar dynamics of the indicator was recorded in patients with HP with normal body reactivity (comparison group). By the end of the observations, there was a tendency to decrease the content of PG

E2 in all three groups. Initially (before surgery), the ratio of PG F2α / PG E2 in the main groups was proportional to that in the first group. Surgical intervention disrupted the balance between these eicosanoid fractions in the main groups and the comparison group. On the 1st day, the ratio of the studied pro- and anti-inflammatory prostaglandins increased, reaching a maximum level on the 2nd day, exceeding the initial values in the main groups, on average, by 1.9 times ($p < 0.05$). This is due to a sharp increase in the content of PG F2α in the blood of patients with generalized periodontitis against the background of a decrease in the concentration of PG E2. Subsequently, there was an equalization of the primary prostaglandin balance - on the 9th day, the ratio of PG F2α / PG E2 reached normal values. As can be seen from this table, the dynamics of the prostaglandin balance index in the main groups against the background of the proposed drug support coincided with that of the body's normoreactivity and was monophasic with a peak on day 2. The increase in the balance index on the 2nd day after surgery testified in favor of a shift in balance towards proinflammatory GH. Subsequently, there was a decrease in the IRPG with the achievement of the initial level on the 6th day and the normal level on the 9th day after flap surgery. This indicates the establishment of a normal balance of eicosanoids in the blood of patients with AP after targeted drug correction. Thus, the proposed differential general and local medication support in patients with generalized periodontitis against the background of hyper- and hyporesponsiveness of the body approximates the values of pro- and anti-inflammatory fractions of prostaglandins to those of normal body reactivity, which ultimately restores their disturbed balance in the blood.

DISCUSSION

Drug treatment of generalized periodontitis is based on the identification of the leading pathogenetic links in the development of the disease [4]. The correct focus of this tactic is confirmed by numerous literature data on the high clinical efficacy of the proposed and implemented drug treatment regimens for generalized periodontitis. This is evidenced by the widespread use of COX inhibitors, immunocorrectors, and cytokine therapy in the complex treatment of generalized periodontitis [7, 8, 10]. However, the issues of clarifying the key mechanisms of the development and course of the disease in each specific clinical case and developing differentiated approaches to the choice of a complex of medications for general and local use, depending on the indicators of the state of the body's reactivity, which have the greatest impact on the pathogenesis of generalized periodontitis, remain relevant. It is known that the intensity and duration of the inflammatory reaction during the healing of a myocardial infarction is determined by the form of the initial reactivity of the body and determines the complicated and uncomplicated consequences [14]. In our study, we have shown that in generalized periodontitis of II and III severity after surgical treatment, the dynamics of prostaglandins (PG) E, E2, F2alpha and cytokines (IL-1β, IL-6, TNF, IL-4) in the blood and gingival fluid is different depending on the initial state of the body's reactivity. Our proposed targeted drug correction in patients with generalized periodontitis against the background of hyper- and hyporeactivity of the body brings the values of prostaglandins and cytokines closer to those of normal body reactivity. As a result, the disturbed balance of indicators in the blood and gingival fluid is restored, which creates optimal conditions for the course of the disease and helps to stabilize the pathological process.

CONCLUSIONS

The complex of drugs for medical support in the treatment of patients with chronic generalized periodontitis should be pathogenetically justified and differentiated depending on the initial state of the body's reactivity, which determines the type of inflammatory reaction and, accordingly, the nature of the healing process and further stabilization of the pathological process. Patients with generalized periodontitis of II, III severity with normal body reactivity should not be prescribed medication correction after surgical treatment. In patients with hyper- and hyporesponsiveness of the body, postoperative drug therapy is appropriate and pathogenetically justified. The complex of means for medical correction should be differential and determined by the nature of the change in the indicators regulating the healing process.

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ORIGINAL ARTICLE

THE ROLE OF LEFT VENTRICULAR HYPERTROPHY, RS1801253 AND RS1801252 ALLELIC POLYMORPHISMS OF ADRB1 IN ASSESSING THE RISK OF SUDDEN CARDIAC DEATH IN PATIENTS WITH ARTERIAL HYPERTENSION

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ABSTRACT

The aim: To study the association of left ventricular hypertrophy (LVH) and polymorphisms rs1801253 and rs1801252 of the ADRB1 gene with the risk of sudden cardiac death (SCD).

Materials and methods: The study included 179 patients which underwent clinical investigation, echocardiography, elektrokardiography. The examined were divided into groups with a low (110 people) and high risk (69 people) of SCD. The distribution of allelic polymorphisms was investigated with polymerase chain reaction (PCR).

Results: All patients of group with high-risk cardiovascular mortality showed a decrease in heart rate variability (RV) due to an increase in sympathetic activity (p=0.013). Also, in the group of patients with LVH, predictors of sudden cardiac death and arrhythmogenic substrate, were observed.

The variability of the allele C1165G rs1801253 of the ADRB1 gene was associated with an increased risk (2.55-fold increase) of SCD and LVH. Also, the associations of polymorphic locus A145G (rs1801252) of the ADRB1 gene proved the presence of a permanent difference for the "risky" allele A in patients with a high risk of SCD.

Conclusions: It was set the probable association of alleles rs1801253 (C1165G) and rs1801252 (A145G) ADRB1 at the patients with a high risk of SCD compared to the control group.

KEY WORDS: sudden cardiac death arterial, gene ADRB1, rs1801253 and rs1801252 allelic polymorphisms, left ventricle hypertrophy

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INTRODUCTION

SCD is one of the most important problems in Public Health, accounting for 20% of total mortality and 50% of cardiovascular mortality in countries of European Union [1]. The 2022 Recommendations of the European Society of Cardiology are currently used as a working document for assessing the risk of SCD [2].

Therefore, the issue of establishing clinical and electrophysiological features, genetic prerequisites of this dangerous condition remains relevant today. A meta-analysis of 20 studies (48,545 participants) showed a direct relationship between the degree of hypertrophy and adverse clinical outcomes (hazard ratio - 2.3) [3] with the development of ventricular tachycardia «torsades de pointes» [4]. The causative genes (or candidate genes) of hereditary arrhythmias have been successfully identified. In one study involving 500 cardiac arrest survivors, a family history of SCD was shown to be an independent risk factor for SCD, increasing its likelihood in 1.75 times [5].

Brodehl A, et al. published the results of a search for copy number variants (CNVs) in genes associated with SCD in a large cohort of patients (n=1765). The frequency of CNVs among SCD cases in patients with LVH was 1.4% [6].

Although the European guidelines for the treatment of patients with coronary heart disease (CHD) do not recommend routine genetic testing of patients (recommendation class – III, level of evidence – C), the study of gene polymorphisms in people with predictors of SCD is quite interesting and relevant. So, the initial ones for arterial hypertension clinical course are such candidate genes, as: ADBR1 (polymorphism of the β1 receptor gene), ADBR2 (polymorphism of the β2 receptor gene), AGTR1 (receptor gene type 1 to angiotensin II) and others [7]. ADBR1 C1165G and A145G β1-adrenoceptor

polymorphisms are being investigated as candidate genes not only for hypertension but also for fatal ventricular arrhythmias [8].

Mutation of the β1-adrenergic receptor (ADRB1) gene (β1-AR) for single nucleotide polymorphisms rs1801253 and rs1801252 results in amino acid substitutions in codons 389 and 49 that affect intracardiac norepinephrine release from adrenergic neurons. No previous study has specifically examined the associations of these different kinds of adrenergic neuroeffector genetic variants with the risk of sudden cardiac death.

THE AIM

The aim was to study the association of LVH and polymorphisms rs1801253 and rs1801252 of the ADRB1 gene with the risk of SCD.

MATERIALS AND METHODS

179 patients treated at the Kyiv Heart Center from 2015 to 2022 were examined. The average age of the patients was 56.8±5.2 years, among them 59.8% (107) were men, 40.2% (72) were women.

The survey included a questionnaire aimed at identifying risk factors for SCD, registration of a 12-channel electrocardiogram, and genetic analysis for the presence of genes associated with LVH.

Based on the questionnaire and ECG data, all the examined were divided into 2 groups: with low (group 1, 110 people) and high risk (group 2, 69 people) of SCD. Group 2 included people with unexplained episodes of loss of consciousness, cases of SCD, hypertrophic or arrhythmogenic cardiomyopathies, long or short QT- syndromes, life-threatening arrhythmias in close relatives.

Preserved LV systolic function was considered when LV EF was more than 60%. All patients underwent 24-hour ECG and BP monitoring using the Card(X)plore device (Meditech, Hungary).

Depending on the severity of LVH, determined by LVMMI, the subjects of the 2nd group were divided into 3 tertiles: 1st tertile - patients with normal LVMMI - 34 people (49.3%) and concentric remodeling of the left ventricle; 2nd tertile - patients with LVMMI >125<150 g/m2 in men and >110<140 g/m2 in women (20 patients, 28.9%), 3-rd tertile - patients with LVMMI >150 g/m2 in men and >140 g/m2 in women (15 patients, 21.8%).

Determination of β1-adrenoreceptor gene polymorphisms (ADRB1) are meant single nucleotide base substitutions (SNPs) in rs1801252 (A145G, Ser49Gly) and rs1801253 (G1165C, Gly389Arg) regions.

Table I. Indicators of heart rate variability in patients at risk of SCD with LVH

Indicators	LVH (n=35)	without LVH (n=34)	p
RR, mc	820,9±87,9	923,7±90,6	<0,001
Amo, %	73,5±10,8	60,6±10,2	<0,001
dRR, mc	142,6±34,8	186,3±29,5	<0,001
SD, mc	25,8±3,45	28,9±4,21	0,335
RMSSD, mc	13,1±4,12	17,0±5,23	0,005
NN50, int	7,7±4,89	12,0±4,56	0,002
pNN50, %	7,1±3,46	17,1±6,12	<0,001
LF/HF	3,8±1,12	2,7±0,78	<0,001
LFnu, un.	69,6±4,12	63,7±4,42	<0,001
HFnu, un.	33,0±6,91	37,2±10,1	0,0236
TP, mc^2	670,2±78,7	700,8±100,6	0,0816
%VLF, %	64,0±7,89	57,1±5,54	<0,001
%LF, %	28,0±5,12	23,4±3,89	<0,001
%HF, %	15,9±4,57	21,0±5,16	<0,001
CV, %	2,73±0,78	3,12±0,81	0,011

Notes: Amo - the number of intervals of the same type; SD - standard deviation; RMSSD - inter-interval differences; NN50 - number of pairs of adjacent R–R intervals that differ by more than 50 ms over the entire recording period; pNN50% is - frequency of rapid rhythm and spectrogram changes; (indicators NN50 and pNN50 reflect the degree of influence of the parasympathetic nervous system on the heart rate); TP - total power of the spectrum; LFnu - power of low frequencies in normalized units, HFnu - power of high frequencies in normalized units, LF/HF - ratio of low and high frequencies; VLF - power of the "very" low-frequency component of the spectrum (slow waves of the 2nd order), the spectral component of the heart rate in the range of 0.04–0.015 Hz (25–65 s); CV % – coefficient of variability of the R–R interval; p - significance of the difference between groups.

Table II. Ventricular extrasystoles (VE) in patients at risk of sudden cardiac death, depending on the degree of left ventricular hypertrophy

Indikator	1 tertile	2 tertile	3 tertile	p
The total number of VE per day	287,2±67,1	384,3±56,9	450,7±62,2	$p_{1,2}$<0,001 p_3=0,002
Average number of single VE per patient	48,9±6,89	55,9±5,75	63,8±7,12	$p_{1,2}$<0,001 p_3=0,009
Average number of paired VE per patient	10,1±4,15	16,2±3,12	21,5±5,76	$p_{1,2}$<0,001 p_3=0,014
Average number of polytopic VE per patient	8,4±3,14	17,4±5,32	25,2±7,82	$p_{1,2}$<0,001 p_3=0,013

Notes: VE – ventricular extrasystoles; p_1 – the probability of the difference between the 1st and 2nd tertiles; p_2 - is the probability of the difference between the 2nd and 3rd tertiles; p_3 - probability between the 1st and 3rd tertiles.
p_1 - significance of the difference between groups 1 and 2; p_2 - significance of the difference between groups 1 and 3; p_3 - significance of the difference between groups 2 and 3.

Table III. Distribution of ADRB1 β1 - adrenoreceptor gene polymorphisms and their correspondence to Hardy-Weinberg equilibrium in patients with low and high risk of sudden cardiac death

Gene	Polymorphism	Genotype	Group 1		p	Group 2		p
			N	%		N	%	
ADRB1 rs1801253	*C1165*G	-1165CC	9	8	<0,05	16	23	<0,01
		-1165CG	57	52		44	64	
		-1165GG	44	40		9	13	
ADRB1 rs1801252	*A145*G	-145AA	10	9	<0,05	18	26	<0,01
		-145AG	40	36		41	59	
		-145GG	60	55		10	14	

p - significance of the difference between groups according to LLχ2.

Table IV. Allele association tests of the polymorphic region rs1801253 of the ADRB1 gene in patients with high and low risk of sudden cardiac death

Allele frequency	Degree of heterozygosity	Degree of homozygosity	Allelic positivity	Test of linear trends (Armitage's test)
		Risk by allele (C)		
[1]<->[2]	[11]<->[12]	[11]<->[22]	[11]<->[12+22]	Common OR
OR=0,49 95%CI=0,30-0,81 χ2=7,77 p=0,005	OR=0,74 95%CI=0,23-2,43 χ2=0,25 p=0,62	OR=0,17 95%CI=0,05-0,64 χ2=7,59 p=0,006	OR=0,49 95%CI=0,16-1,57 χ2=1,48 p=0,22	OR=0,38 χ2=11,4 p<0,001
		Risk by allele 1 (G)		
[2]<->[1]	[22]<->[12]	[22]<->[11]	[11+12]<->[22]	Common OR
OR=2,02 95%CI=1,23-3,33 χ2=7,77 p=0,005	OR=4,27 95%CI=1,86-9,77 χ2=12,58 p=0,003	OR=5,77 95%CI=1,56-21,28 χ2=7,59 p=0,006	OR=4,46 95%CI=1,98-10,05 χ2=14,16 p<0,001	OR=2,55 χ2=11,4 p<0,001

p - significance of the difference between groups according to χ2.

For the statistical analysis was used "Statistica for Windows" 10.0 (Stat Soft Inc., USA). Values are presented in the form of frequencies (percentage of observations to the total number of examinees) and means (numerical data) with standard deviation (M±SD). For comparing the numerical data, the one-way ANOVA with post-hoc analysis was used. LL(log-likelihood) χ2, Armitage's test for linear trends (common OR) were used to compare discrete values in independent samples. At p<0.05, differences were considered statistically probable.

RESULTS

All patients of group 2 showed a decrease in heart RV due to an increase in sympathetic activity (p=0.013) and a decrease in vagal influences (p=0.037). However, in the group with LVH (Table I), there was a significant decrease in the integral indicator of heart RV - the standard deviation of R–R intervals (SD, p<0.05), parameters characterizing parasympathetic influences (dRR, pNN50, p<0.05), and the total power of the spectrum (TR,

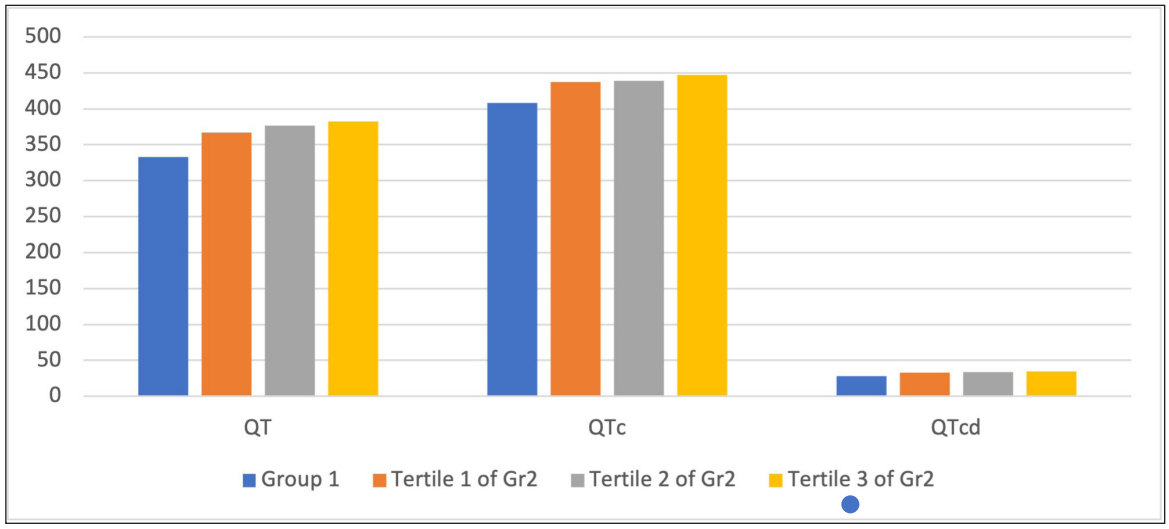

Fig. 1. Duration of average daily QT, QTc, QTcd intervals.
Notes: ● - the probable difference between groups.

Table V. Association tests of alleles of the polymorphic region rs1801252 of the ADRB1 gene in patients with a high risk of sudden cardiac death

Allele frequency	Degree of heterozygosity	Degree of homozygosity	Allelic positivity	Test of linear trends (Armitage's test)
		Risk by allele 2 (A)		
[1]<->[2]	[11]<->[12]	[11]<->[22]	[11]<->[12+22]	Common OR
OR=1,77 95%CI=1,18-2,65 χ2=7,61 p=0,006	OR=3,05 95%CI=1,59-5,6 χ2=11,60 p=0,006	OR=3,32 95%CI=1,17-9,44 χ2=5,32 p=0,02	OR=3,09 95%CI=1,63-5,84 χ2=12,48 p<0,001	OR=1,98 χ2=10,04 p=0,002
		Risk by allele 1 (G)		
[2]<->[1]	[22]<->[12]	[22]<->[11]	[11+12]<->[22]	Common OR
OR=0,57 95%CI=0,38-0,85 χ2=7,61 p=0,006	OR=0,92 95%CI=0,35-2,42 χ2=0,53 p=0,86	OR=0,30 95%CI=0,11-0,86 χ2=5,32 p=0,02	OR=0,64 95%CI=0,25-1,63 χ2=0,89 p=0,35	OR=0,49 χ2=10,04 p=0,002

p<0.05), as well as an increase in indicators reflecting sympathetic influences (Amo, LF HF, p<0.05). Vegetative imbalance in patients with LVH was more pronounced (p<0.05), mainly due to a decrease in vagal activity.

Thus, in the group of patients with LVH, predictors of SCD, which characterize the presence of an arrhythmogenic substrate, were more often observed.

The average value of LVMMI in all examined group 2 was 245.1±14.2 g, LVMMI - 122.4±25.1 g/m2. At the same time, LVMMI was 118.6+18.8 in the first tertile, 131.7+24.0 in the second, and 156.9+28.2 g/m2 in the third (p<0.001). In this group, significant differences were found between tertiles in the total number of ventricular extrasystoles (VE) per day, the average number of paired VE per patient, the average number of polytopic extrasystoles, Table II.

In regression analysis, a direct, medium degree of dependence and a reliable relationship between LVMMI and the total number of VE per day (r=0.407; p=0.033), the average number of paired VE (r=0.588; p=0.021), the average number of polytopic extrasystoles (r=0.503; p=0.045) were revealed.

The Groups of patients did not reliably differ between tertiles in terms of average daily intervals of QT, QTc, QTcd according to the criterion of multiple intergroup comparison (p=0.68, p=0.73, p=0.73, respectively). At the same time, these indicators in group 2 were significantly higher than in group 1 (p=0.024, p=0.032, p=0.024), fig. 1.

The next task was to evaluate the distribution of allelic polymorphisms C1165G and A145G of the ADRB1 β1 gene - adrenoreceptor in patients with low and high risk of SCD. At the initial stage, the distribution of polymorphisms of the rs1801253 and rs1801252 loci was studied, respectively, of the Hardy-Weinberg equilibrium in groups 1 and 2. The results are presented in Table III.

Analyzing the obtained data, it should be noted that there is no statistical deviation from the Hardy-Wein-

berg equilibrium in the control group. In contrast, the distribution of genotypes of group 2 polymorphisms for ADRB1 rs1801253 (C1165G) and rs1801252 (A145G) genotypes was characterized by a probable deviation from the Hardy-Weinberg equilibrium.

Conducting a monolocus analysis of the associations of alleles and genotypes of the corresponding polymorphisms with the risk of SCD confirmed the presence of changes between group 1 and group 2. Further, the determination of the association between allelic polymorphisms and the risk of SCD in patients was carried out using an additive model of inheritance with calculation of the final common Odds ratio after conducting the Cochran-Armitage test for linear trends. Association tests of the C1165G rs1801253 allelic polymorphism of the ADRB1 gene are presented in Table IV.

Based on the analysis of the distribution of alleles of the polymorphic locus rs1801253 of the ADRB1 gene, it was established that there is a probable difference in the distribution of frequencies in patients with a high risk of SCD compared to the control group. The variability of the allele C1165G rs1801253 of the ADRB1 gene was associated with an increased risk (2.55-fold increase) (Table 4) of SCD and LVH in patients of group 2 compared to group 1. In addition, group 2 of patients differed significantly in the level of heterozygosity (OR=4.27; 95% CI = 1.86 9.77; $p<0.05$) from the control (which, in principle, explained the deviation of the frequency of occurrence of genotypes from the Hardy-Weinberg equilibrium). In addition, a decrease (by 4.46 times) in the detection frequency of the protective allele -1165G was observed in the group of patients with a high risk of SCD.

The analysis of associations of alleles of the polymorphic locus A145G (rs1801252) of the ADRB1 gene proved the presence of a permanent difference for the "risky" allele A in patients with a high risk of SCD using an additive model for a linear trend, the odds ratio (OR) is 1.98 ($p=0.002$), Table V.

The model is characterized by an increased degree of heterozygosity and the presence of allelic positivity in the main group (3.3 and 4.8 times, respectively, at $p<0.001$).

Thus, according to the study, in the group with a high risk of SCD, a high frequency of occurrence of homozygous variants C1165C (rs1801252) and A145A (rs1801252) of the ADRB1 gene, as well as heterozygous variants C1165G (rs1801252) and A145G (rs1801252) was revealed. That is, polymorphisms responsible for the regulation of norepinephrine production in the myocardium and the expression of β1-adrenergic receptors, as well as LVH. Correlation analysis revealed a direct significant correlation relationship between the presence of episodes of SCD in relatives under the age of 50 and the number of homozygous variants ($r=0.47$; $p=0.009$) with C1165C polymorphism (rs1801252) and A145A polymorphism (rs1801252) ($r=0.52$; $p=0.007$).

DISCUSSION

Regardless of the etiology of hypertrophy, the risk of SCD in such patients increases. The REGARDS Study showed an independent association between LVH and SCD in patients with risk factors for CHD [9]. Another long-term prospective study found that LVH increased the likelihood of SCD independently of other coronary risk factors [10].

Several studies have examined the influence of the ADRB1 polymorphisms on cardiovascular events and all cause mortality in CAD patients. For example, Amare AT. et al. has proved, that there were the increased the number of cardiovascular side effects in the middle of adopting one CG allele to the ADRB1 rs1801253 polymorphism, and lower the middle of GG homozygotes [11]. However, Li et al. (2013) did not reveal an association between ADRB1 rs1801253, ADRB2 rs1042713 and ADRB2 rs1042714 polymorphisms and CVDs in Chinese patients [12]. A study by C. Iwai in Japan among post-MI patients showed an increase in the clinical success rate in GG homozygotes compared to CG heterozygotes [13]. At the same time, in the study by S.M. Wallerstedt did not find a relationship between the carriage of ADRB1 rs1801253 alleles and SCD [14].

It has been shown that patients with coronary atherosclerosis with Gly49Gly polymorphism or its combination with Arg389Arg had a significantly lower rate of hospitalization. According to Chevalier P. et al., a decrease in mortality and hospitalization rates in patients with chronic heart failure was observed in patients with the Gly49Gly haplotype, compared with the same patients, but with the Ser49Ser haplotype [8].

CONCLUSIONS

In our study was investigated the distribution of functionally important polymorphisms rs1801252 and rs1801252 of the ADRB1 gene in patients with high risk of SCD. Risk of SCD was determined on the basis of a questionnaire, family history, clinical and electrocardiographic data. All patients of group with high-risk cardiovascular mortality showed a decrease in HR variability due to an increase in sympathetic activity ($p=0.013$). Also, in the group of patients with LVH, predictors of SCD, which characterize the presence of an arrhythmogenic substrate, were observed.

Due to monolocus analysis of the associations of alleles rs1801253 (C1165G) and rs1801252 (A145G)

ADRB1 was established the probable difference in the distribution of frequencies in patients with a high risk of SCD compared to the control group. The variability of the allele C1165G rs1801253 of the ADRB1 gene was associated with an increased risk (2.55-fold increase) of SCD and LVH. Also the associations of polymorphic locus A145G (rs1801252) of the ADRB1 gene proved the presence of a permanent difference for the "risky" allele A in patients with a high risk of sudden cardiac death.

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ORIGINAL ARTICLE

REFERRING PHYSICIANS' KNOWLEDGE OF THE RADIATION DOSES FOR COMMONLY RADIOLOGICAL INVESTIGATIONS IN NASIRIYA TURKISH HOSPITAL

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ABSTRACT

The aim: To assess the referring physicians' knowledge of the radiation doses for commonly radiological investigations in Nasiriya Turkish Hospital (NTH).

Materials and methods: A cross-sectional study of referring physicians in NTH was carried out. A two-part questionnaire was distributed to all Referring Physicians' apart from radiologists. Radiological examinations were listed and Referring Physicians' were asked to estimate equivalent doses using the dose of postero-anterior chest X-Ray as a reference. Questions on knowledge of radiation (including radiation exposure and doses, protection, maximizing effectiveness, impacts of ionizing radiation) and utilization of referral guidelines were included.

Results: A total of 50 were distributed to the participants, 32 were returned and deemed acceptable for inclusion in this study. The participants' demographic data shows that most are male 21 (65.6%), while 11 (34.4%) are female. Up to 23 (71.9%) of participants had knowledge of ionizing radiation, but only 7 (21.9%) physicians were aware of its unit measurement. Most participants were unable to accurately estimate the radiation dose absorbed by patients during the various radiological examinations; 68.8% had no knowledge of radiations' stochastic and non-stochastic impacts; 14 (43.7%) were aware of the referral guidelines and used them. The majority of the participants 24 (75%) are aware that USS is safe for pregnant women. only 1(3%) physician knew that there is no ionizing radiation involved in MRI.

Conclusion: Most participants did not able to accurately estimate the radiation dose absorbed by patients during the various radiological examinations.

KEY WORDS: referring physicians, radiological investigations, radiation dose

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INTRODUCTION

Radiation dose refers to the quantity of energy accumulated in a medium [1]. The radiation dose is managed in a variety of manners by diverse X-Ray modalities. Radiation doses in radiography and mammography are given by the entrance skin dose and average glandular dose, respectively [2]. The radiation dose in computed tomography (CT) is conveyed by the computed tomography dose index, which measures the radiation quantity the patient receives in CT. Ideal phantom model testing (32 cm/16 cm water phantom to represent the average adult/pediatric patient) is the basis of dose estimates utilizing computed tomography dose index (CTDI) or dose length product (DLP) [3]. The most prevalent and expedient means of indirect dose monitoring in fluoroscopy is to use a dose area product meter [4]. Increasingly, the diagnoses of a broad spectrum of diseases and injuries are being facilitated by diagnostic imaging and interventional radiology, and in many cases, these techniques are also providing life-saving treatments [5]. Since its introduction, radiation has undergone a huge evolution, and currently, 30-50% of medical decisions are informed by radiological examinations [6]. However, it remains constrained by the risks it entails for both patients and healthcare providers [7]. Radiological examinations are pivotal in medicine and typically, physicians from a wide variety of clinical environments refer their patients for radiological investigations. The existing literature reports that doctors generally have insufficient radiation knowledge [8]. Another observation is that when a patient is referred, the radiation dose they receive is 16 times greater than most doctors realise, and the average irradiation dose is six times larger than they predicted [9]. A radiological investigation is a core component of diagnosing a condition and constructing a patient management plan in the vast majority of specialities. Radiology encompasses many elements (such as an interventional endovascular treatment or a staging CT in oncology) and is a vital area of medicine [10]. During any diagnostic procedure, the radiation dose should simultaneously be sufficient to provide insight into the

clinical issue and low enough to minimize the risk to the patient. Contemporary imaging equipment can be altered to suit the size and anatomy of the patient. This is essential because there is a significantly higher lifetime associated fatal cancer risk following radiation exposure to children than to adults [7]. CT doses are the greatest of all radiological examinations [11]. The usual exposure doses for abdominal CTs and chest radiographs are 9 mSv and 0.02 mSv, respectively [12]. Therefore, it is important that due consideration is given when employing ionizing radiation-based methods; a risk/benefit analysis should be conducted, other approaches should be used where possible, and the minimum effective dose to address the clinical issue should be administered [13]. The average annual dose of radiation received by the public is 2.5 mSv, of which 15% is linked to medical exposure [14]. The detrimental biological impacts of radiation, which fluctuate depending on dose and exposure duration, are reported; hence, it is critical that physicians have comprehensive knowledge and awareness of the risks associated with it. As per the ionizing radiation (medical exposure) regulations, physicians have a certain level of responsibility in this area, due to the fact that they are referring the patients for these examinations [15]. Employee behavior is affected by the extent of their awareness of radiation protection [16]. Inadequate knowledge of radiation safety can lead to unsafe behaviors and consequently, unfavorable outcomes [17]. Aside from implementing new and improved policies and procedures, the issue at hand is to establish a stronger comprehension of the causes and rates of mistakes, especially those that have a higher probability of resulting in damage [18]. Whilst acknowledging the advantages and significance of radiological imaging in medical diagnoses, it is also critical to raise the level of awareness of the risks associated with ionizing radiation. Furthermore, it is vital that physicians are well trained in diagnostic imaging that entails ionizing radiation and understand the related risks. This is of particular importance in health structures, in which numerous radiological imaging examinations are conducted daily, typically in an atmosphere where time is limited.

THE AIM

This study's aim is to assess the referring physicians' knowledge of the radiation doses for customary radiological investigations in Nasiriya Turkish Hospital (NTH).

MATERIALS AND METHODS

Following approval from the Ethical Review Board, a cross-sectional study of referring physicians in NTH was carried out. This study employed convenience sampling, which means the participants were chosen according to their willingness to take part and their availability.

The inclusion and exclusion criteria were as follows:

- Inclusion: all referring physicians in NTH;
- Exclusion: all radiological doctors and physicians external to NTH.

A two-part questionnaire was distributed to the participants to collect the data, which was designed in accordance with the study objectives. Part 1 collects information about the participants' demographics (e.g., age, gender, qualifications, and experience in years). Part 2 assesses the participants' fundamental knowledge of radiation (including radiation exposure and doses, protection, maximizing effectiveness, impacts of ionizing radiation) and their utilization of referral guidelines. Once the questionnaires were returned and deemed acceptable for inclusion in this study, the data was organized and classified. Subsequently, data analysis was conducted via the statistical package for social sciences (SPSS) version 23.0. Descriptive statistics were used to evaluate the variable distribution [18].

RESULTS

The demographic distributions of the participants is illustrated in table I. It shows that the majority of the participants were male (64.6%), those with age ranging between (25-30) years (87.5%), those working as residents doctors (75%), and 27(84.4%) have between 1 and 5 years of experience.

Table I. Demographic characteristics of the study participants.

Parametr	Frequency	Percentage
Gender		
Male	21	65.6%
Female	11	34.4%
Age [years]		
25-30	28	87.5%
31-40	3	9.4%
41-50	1	3.1%
Qualification		
Consultant	2	6.25%
Resident	24	75%
Medical officer	2	6.25%
House officer	4	12.5%
Time of experience [years]		
1-5	27	84.4%
6-10	3	9.4%
>10	2	6.2%

Table II. Basic radiation knowledge of study participants

Questions	Correct response [%]	Wrong response [%]
Definition of radioactive radiation	71.9	28.1
Unit of Radioactivity	21.9	78.1
Annual Radiation dose limit to patients	3.1	96.9
A person annual radiation dose from natural background radiation	6.3	93.7
Radiation dose of a single chest X-Ray	15.6	84.4
Aware of the ALARA principle	40.6	59.5

Table III. Awareness of imaging referral guidelines.

Responses	Frequency	Percentage [%]
YES, and used it	14	43.7
NO	18	56.3
Total	32	100

The basic radiation knowledge among participants is shown in table II. It reveals that 71.9% of the participants have correct answers regarding "Definition of radioactive radiation", about 21.9% of the participants have correct answers regarding "Unit of Radioactivity", and only 3.1% of them have correct answers concerning "Annual Radiation dose limit to patients", approximately 6.3% of them have correct answers about "A person annual radiation dose from natural background radiation", about 15.6have correct answers concerning "Radiation dose of a single chest X-ray", and finally about 40.6% have correct answers regarding "Aware of the ALARA principle".

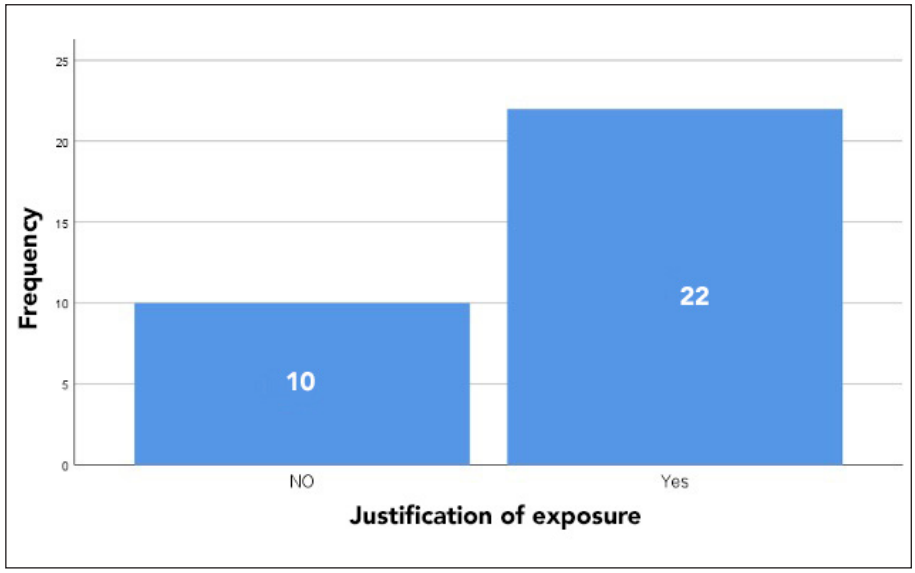

Fig. 1. Justification of exposure in relation to any alternatives.

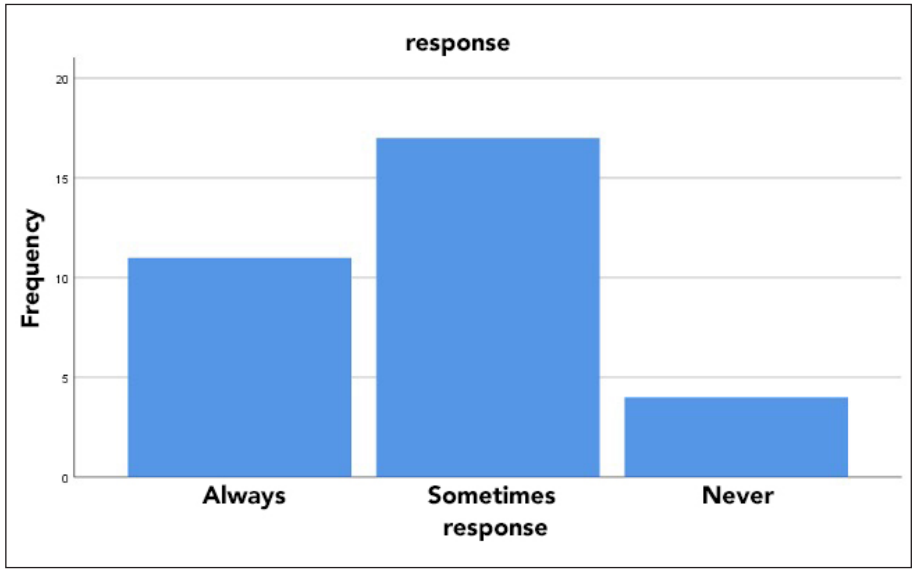

Fig. 2. Inform patients of the risks and benefits of radiation exposure.

Table IV. The rate of physicians' estimations of the dose of radiation absorbed by patients undergoing radiological examinations, with the chest X-Ray radiation dose as a reference.

Type of exam	Under-estimate [%]	Correct estimate [%]	Over-estimate [%]	Do not know [%]
Plain abdomen	46.8	6.2	0.0	46.8
Pelvis	34.3	15.6	3.1	46.8
Lumbar spine	40.6	9.3	0.0	50
IVU	37.5	3.1	0.0	59.3
MRI brain	0.0	34.3	18.7	46.8
Abdominal USS	0.0	37.5	12.5	50
CT abdomen	34.3	15.6	0.0	50
Mammography	37.5	3.1	0.0	59.3

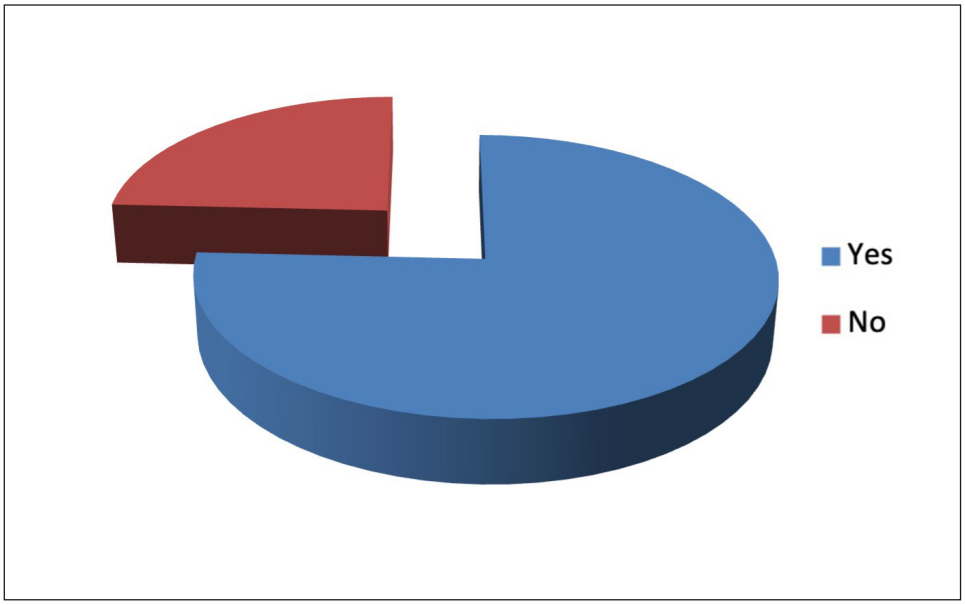

Fig. 3. Awareness about effects of radiation.

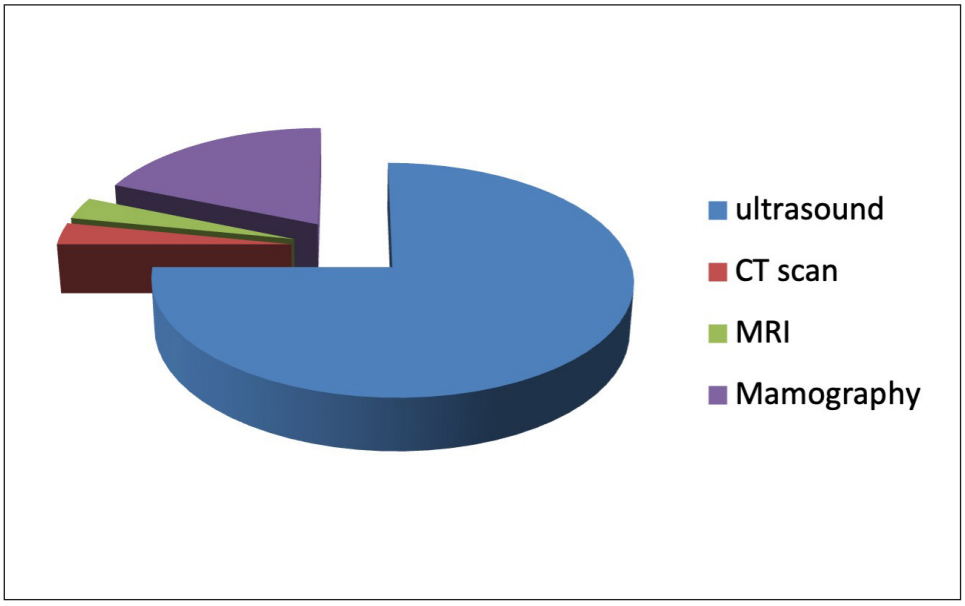

Fig. 4. Awareness about none ionizing nature of Magnetic resonance imaging (MRI) and ultrasound scan (USS).

According to results of the 32 participants, 22 (68.7%) expressed that they felt justified in referring patients for an examination rather than pursuing an alternative course of action, whereas 10 (31.2%) did not (Fig.1).

Regarding to obtained results of the 32 participants, 17 (53%) sometimes inform patients about the risks and benefits of exposure when obtaining consent for the examination entailing radiation, 11 (34%) do so every time, and 4 (13%) never do this (Fig. 2).

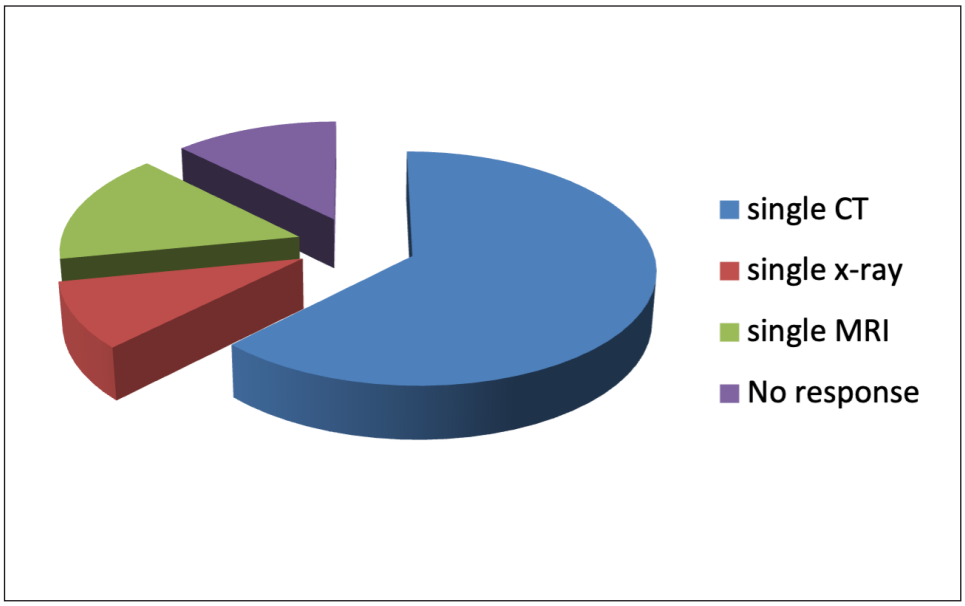

Fig. 5. Awareness about modality with the highest radiation dose.

Regarding to awareness of imaging referral guidelines, it is shown that of the 32 participants, 14 (43.7%) expressed that they are aware about imaging referral guidelines and they use it, whereas 18 (56.3%) did not (Table III).

Concerning awareness about effects of radiation it is shown that of the 32 participants, only 10 (31%) had an awareness of the stochastic and non-stochastic radiation effects, while 22 (69%) had no knowledge of this (Fig. 3). Moreover, only 7 (22%) were able to categorize the potential harmful impacts of radiation as stochastic or non-stochastic.

According to our results, it is shown that of the 32 participants, 24 (75%) were aware that ionizing radiation is not a component of USS, but only 1 physician (3%) knew that it is not involved in MRIs (Fig. 4).

Regarding to results of research, it is shown that of the 32 participants, 20 (62.5%) were aware that a CT carries the greatest radiation dose, while 5 (15.6%) thought it was a single X-Ray and 3 (9.4%) opted for a single MRI. The remaining 4 (12.5%) participants did not answer this question (Fig. 5).

Most participants were unable to accurately estimate the radiation dose absorbed by patients during the various radiological examinations, either underestimating the dose or answering: 'do not know' (Table IV). The table also shows that about (34.3%) have correct estimate regarding MRI brain, (37.5%) have correct estimate concerning (Abdominal USS).

DISCUSSION

The participants' demographic data in the current study are in accordance with the research conducted in Nigeria by Ahidjo et al. [19]. The reason for this is that it is the norm in most hospitals in Iraq that resident doctors are always on the wards. This current study determined that while 23 (71.9%) participants had knowledge of ionizing radiation, only 7 (21.9%) were aware of its unit measurement. This finding is also in line with Ahidjo et al. [19], who established that only 38.5% of physicians could identify the radiation unit. Furthermore, only 13 (40.6%) participants knew the ALARA principle. Another study conducted by Hobbs et. al. [11] has shown that, the participants' knowledge about radiation exposure and its associated risks, especially regarding various imaging techniques, was quite limited. Notably, 26% of the participants were unable to correctly identify the imaging modalities that expose patients to ionizing radiation. According to the same study, radiologists had higher scores before the presentation compared to other specialties, indicating they had less room for improvement. Nevertheless, even radiologists showed enhancement in their knowledge of radiation safety after the educational session. Among the other specialties, there was no significant difference in their initial understanding of radiation exposure and risk. This was supported by Gour et al. [20], who found that only 27% of physicians in India could identify this, despite the fact that this principle is the foundation of the philosophy of radiation protection. This is a serious issue, particularly when high doses of radiation are used repeatedly as screening instruments to assess the progression of diseases. The present study also revealed that there is a substantial lack of awareness amongst the participants about the radiation dose received by patients undergoing the radiological investigations that have been discussed. The majority either underestimated the dose or selected the 'do not know' answer option. In reality, the dose estimations for some radiological

examinations are far below the actual dose, and this finding is supported by the existing literature. There is a risk that this underestimation of the ionizing radiation dose could lead doctors to refer patients for radiological examinations more frequently than is required or is safe to do so. The findings show that only 11(34%) of the participating physicians always explain the risks and benefits of exposure to radiation to their patients when seeking consent for radiological examinations. This is in accordance with Gour et al. [20], who reported that 38% of physicians always inform their patients. Frequently, patients referred for examinations such as abdominal CTs have not been adequately informed about the risks associated with the procedure. This is likely attributable to physicians own lack of knowledge about radiation doses and their potential detrimental impacts. Of the 32 physicians participating in this study, only 1 (3%) knew that there is no ionizing radiation involved in MRIs. Ahidjo et al. [19] reported a higher rate of 14% for this question. This disparity demonstrates that physicians in more efficient developing nations (such as Nigeria) have greater knowledge in this area than physicians in less efficient developing nations (such as Iraq). In this study, this result is possibly due to the dearth of MRI specialists and equipment in Iraq. In contrast, the majority of the participants (24, 75%) are aware that USS is safe for pregnant women. This is in line with the studies conducted by [11, 12], Turkey with rates of 95% and 96%, respectively. This indicates that most physicians are suitably informed that ionizing radiation is not involved in USS. This study also found that 14 (43.7%) participants were aware of the referral guidelines and used them, whereas 6 (18.7%), whilst also aware, did not use them. *Ahidjo et al.* [19] reported comparable outcomes (41.4%). Most of the participants 22 (68.8%) had no knowledge of radiations' stochastic and non-stochastic impacts, meaning that only 10 (31.3%) were aware. Of this, only 15.6% could correctly categorize the listed potential impacts of radiation as stochastic or non-stochastic. This indicates the lack of knowledge amongst most physicians about the harmful effects of radiation exposure to the human body. The research conducted by *Ahidjo et al.* [19] also highlighted this lack of awareness of radiation exposures' impact on the body, reporting that only 23.1% of the participating physicians could accurately categorize radiation injuries as stochastic or non-stochastic [5]. Comparing against other studies on this topic, it was found that physicians in the UK, Turkey, and India have greater knowledge about MRIs not involving ionizing radiation than physicians in Iraq.

CONCLUSION

The majority of participants have decreased knowledge regarding basic radiations especially that related to annual radiation dose limit, a person annual radiation dose, and radiation dose of a single chest X-Ray. Most participants were not aware about imaging referral guideline, and they didn't use them, they are also not able to accurately estimate the radiation dose absorbed by patients during the various radiological examinations.

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ORIGINAL ARTICLE

DIFFERENTIAL-DIAGNOSTIC INFORMATIVENESS OF THE MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF PAROTID SALIVARY GLAND CYSTS

DOI: 10.36740/WLek202309124

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ABSTRACT

The aim: To conduct a general analysis of the results of the study of the morphological and immunohistochemical structure of cysts of the parotid salivary glands.
Materials and methods: Our study is based on the application of generally accepted, additional and special methods of examination, which concerns 21 patients who underwent surgical intervention to remove cystic formations of the parotid salivary gland.
Results: It was established that there are 2-3 HLA-DR+ cells per 100 epithelial cells located in the basal and subbasal layers in the form of their continuous ribbon and their moderate infiltration of tissues within the acinar epithelium. In the epithelium, CD3+ cells were also detected in the number of 1 to 7 per 100 epitheliocytes and they were the most numerous, along with HLA-DR+ cells. Instead, the presence of CD4+ and CD20+ cells was not detected in the epithelium, unlike the subepithelial layer, where they occupied significant areas. In turn, the infiltration of CD8+ cells of the epithelial layer was established in the amount from 1 to 7 per 100 epitheliocytes. A moderate number of them was also determined subepithelially, and they were single directly in the cyst wall.
Conclusions: Immunohistochemical study of the structural components of cystic formations is this is the direct way to establish the nature of the redistribution of immune cells in it, which is very important when conducting differential diagnosis in difficult and doubtful cases.

KEY WORDS: parotid salivary gland, cyst, morphological structure, immunohistochemistry

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INTRODUCTION

Cystic formations more often originate from the small salivary glands (56%), less often from the sublingual (35%), parotid (5%) and submandibular (4%). They develop mainly in young and middle-aged people, and their formation may be associated with a partial or complete cessation of the outflow of secretion. The leading causative factors of impaired patency of the ducts of the glands can be their blockage with a mucous plug, obliteration due to the presence of an inflammatory process both in the gland itself and in the case of direct traumatic damage to it and adjacent soft tissues, obstruction by a stone of the duct system, cicatricial narrowing or external compression by a tumor. There are opinions that some cysts of the large salivary glands can develop during embryogenesis and originate from the epithelium that was detached or formed from an additional rudimentary duct, therefore, nowadays, the questions of their etiology and pathogenesis remain debatable [1, 2].

Most often, cysts of large salivary glands are recognized by the clinical picture of their development, but in order to establish the correct diagnosis, it is necessary to carry out additional differential diagnosis using special research methods. Ultrasound examination, cystography, sialography, MRI in the contrast mode is usually performed to clarify the size, structure and position of the cyst, its connection with the salivary gland, especially when there is a suspicion of malignancy of the formation. An aspiration puncture biopsy with subsequent biochemical and cytological examination of the cellular composition of the contents and a study of the morphological structure of the cyst itself after its removal is of great importance in confirming the diagnosis [3-5].

Cysts of the parotid salivary glands are always represented by cavity formations that arise as a result of obturation of their excretory ducts and are filled with liquid contents. Clinically, they are manifested by the presence of a dense or dense-elastic painless protrusion, a slow

increase in size, and sometimes can lead to difficulty in swallowing. Diagnostic measures involve the use of a classic version of generally accepted methods, which include the collection of complaints, medical and life anamnesis, analysis of data from visual, clinical, additional and special examination methods [6, 7].

However, it should be noted that the classic method of histological verification of parotid gland cysts is sometimes controversial, and fine-needle aspiration cytology (FNA-fine-needle aspiration), which is most often used and is associated with a high frequency of false-negative results. In such cases, the use of coronal biopsy under ultrasound control is a more invasive procedure than FNA, but safer and more diagnostically effective. In turn, the frozen biopsy section provides better species specificity than FNA, but it also has a number of disadvantages and cannot be considered as the main diagnostic tool. Therefore, recently, more and more attention of scientists is paid to the newest methods, which include the immunohistochemical variant of studying the layers of cystic membranes, which determines the relevance of this scientific research [8].

THE AIM

The aim of the research is to conduct a general analysis of the results of the study of the morphological and immunohistochemical structure of cysts of the parotid salivary glands.

MATERIALS AND METHODS

Our study is based on the application of generally accepted, additional and special methods of examination, which concerns 21 patients who underwent surgical intervention to remove cystic formations of the parotid salivary gland on the basis of the maxillofacial surgery department of the ME "Poltava Regional Clinical Hospital named after M.V. Sklifosovsky" of Poltava Regional Council in the period from 2015 to 2022. During the examination of the patient, the size, shape of the cyst, turgor, color of the skin in the area of its location, the presence of an inflammatory reaction, consistency, reaction of the regional lymph nodes were evaluated.

With the help of a ultrasound-controled puncture biopsy, material was obtained from the formation for further study of the cellular composition of the punctate after staining the smear according to Romanovsky-Giemza with the determining of the activity of α-amylase in it, and the presence of which allows to establish the fact of the presence of a cyst of the salivary gland, and this was carried out according to the Karavey`s method. Ultrasound examinations were carried out using scanners "HDI 5000", "Dornier AI 5200", "Aloka 630", with linear or convex sensors and a radiation frequency of 7.5 or 10 MHz. Multifrequency hydrogel acted as an intermediate medium. In tricky cases and when there were doubts or suspicions about the malignancy of the formation, 5 patients underwent an MRI examination according to the standard method.

Sections for studying the morphological structure of the cyst shell with a thickness of 6-8 μm were prepared from 21 blocks on a MBS-2 microtome using standard classical methods. They were fixed on glass slides and stained with hematoxylin-eosin. Van Gieson's picrofuchsin staining was also applied and the PAS-reaction was performed. [9, 10]. The results were documented using a light microscope "Olympus BX-41" (Japan) with a photomicroscope and a set of licensed image processing programs. Part of the results were captured by filming with a microscope using a video camera "Panasonic WV-CP410/ G" (Japan) on the basis of the department of pathological anatomy of the National Medical Academy of Postgraduate Education named after P.L.Shupyk.

In addition, biopsies of the cyst walls were placed in a 6% solution of carboxymethylcellulose (Sigma, USA), which was frozen in liquid nitrogen, and sections 5-7 μm thick were made on a microtome-cryostat. The immunohistochemical study consisted in the determination of subpopulation markers of immunocytes localized in different layers of 21 shell biopsies. At the same time, HLA-DR+, CD3+, CD4+, CD8+, CD20+ immune cells were studied for quantitative and qualitative characteristics, using monoclonal antibodies to these molecules produced by "Sorbent". Immunohistochemical study on cryostat sections was carried out according to the previously described method [11-14].

In order to determine the shares in percentages, a generally accepted statistical method was used [15].

RESULTS

Of the 21 patients selected for further in-depth research, 18 patients (85.7%) were referred by communal city polyclinics, and the remaining 3 (14.3%) were referred by primary care institutions. At the pre-hospital stage, dental surgeons diagnosed 17 people (80.9%) as acute or chronic lymphadenitis of the submandibular region, and only 4 patients (19.1%) had a diagnosis corresponding to the nosological form of the disease.

Regarding the duration of the disease, the patients presented ambiguous data: 9 patients (42.8%) noted that the onset of the disease reaches more than 1 year,

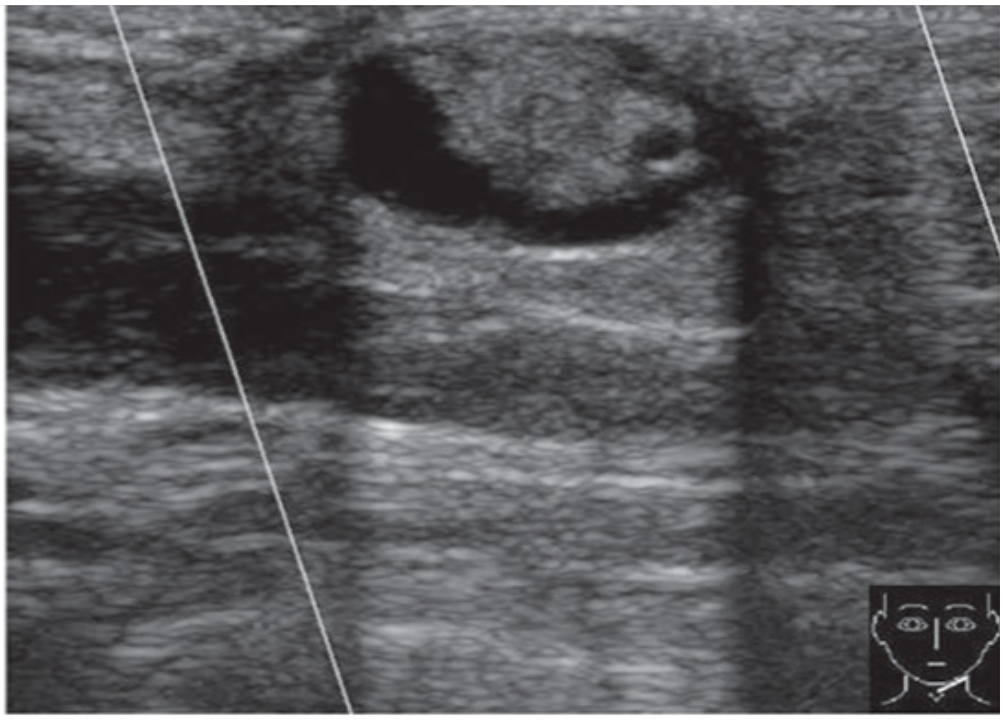

Fig. 1. Echogram of the parotid salivary gland. The presence of a cystic formation measuring 3x4 cm in the lower pole of the gland with hypoechoic content and finely dispersed inclusions was determined.

Fig. 2. Image of a parotid salivary gland cyst on an MRI slice. Within the location of the lower pole of the gland, a formation measuring 2x2 cm is visualized.

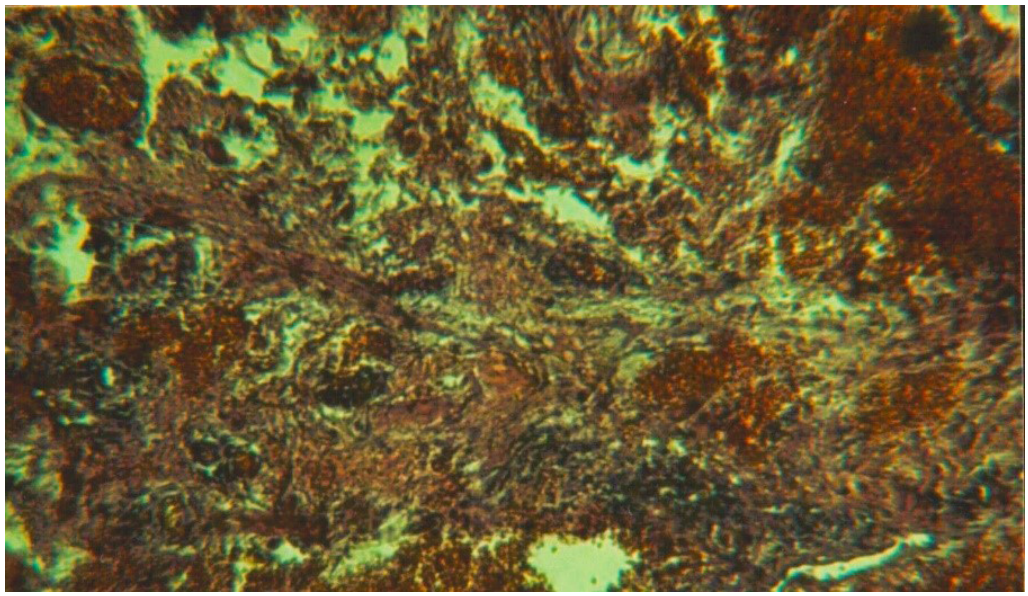

Fig. 3. Positive PAS-reaction throughout the thickness of the epithelial layer of the cyst.

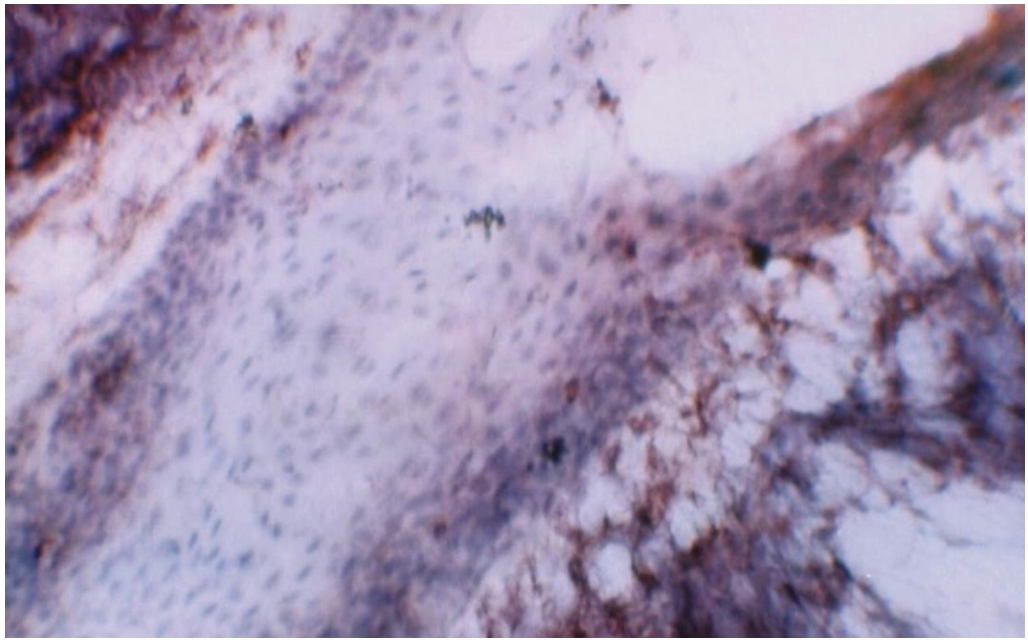

Fig. 4. The presence of HLA-DR+ cells within the acinar epithelium.

7 patients (33.4%) indicated a period of 6 months, 3 patients (14.3%) indicate that the formation appeared about 3 months ago and 2 patients (9.5%) noted the appearance of swelling no more than a week ago. The vast majority of patients sought medical help from specialists of related profiles at their place of residence, and usually they were prescribed for symptomatic treatment.

During the visual assessment of the condition of the maxillofacial region of the patient, asymmetry was determined due to the presence of a tumor formation in the upper third of the lateral region of the neck. In most patients (14 people, 66.7%) the formation was oval in shape, and in 7 patients (33.3%) it was rounded. The skin above the swelling was of a normal shade and it was taken in a strip.

Variation in the size of the cysts was also noted: in 15 patients (71.4%), they were from 2 to 4 cm, in 6 cases (28.6%) - from 4 to 5.5 cm. We determined the consistency of the formation in 1 case (4.8%) as dense, in 17 patients (80.9%) as dense-elastic, and in 3 (14.3%) as elastic. During the palpation examination of the cysts, the contour surface was smooth in all of them, the formations were painless, not fused with the surrounding tissues, but slightly limited in mobility. The reaction of regional lymph nodes was determined in 11 patients (52.4%) both by palpation and during ultrasound examination.

According to the results of an ultrasound examination, these formations had clear contours of a hyperechoic shell with a thickness of 1-2 mm, as well as a hypoechoic structure and finely dispersed inclusions (Fig. 1).

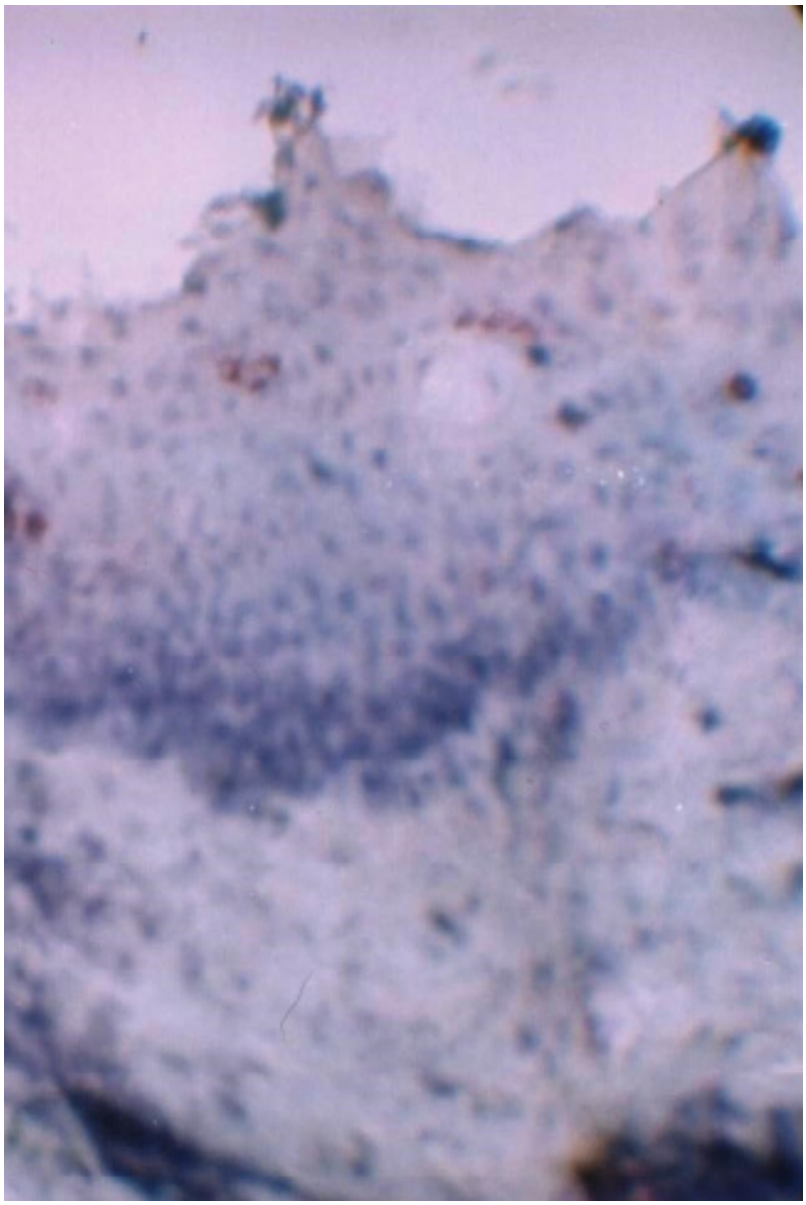

Fig. 5. Presence of CD3+ cells in the epithelial layer.

During fine-needle aspiration under ultrasound control, a transparent liquid of a slightly yellowish color with mucus impurities was obtained. Cytological examination of the punctate revealed the presence of a large number of flat epithelial cells in the form of layers and stratums, single erythrocytes, lymphocytes and cholesterol crystals against the background of structureless masses. The result of the biochemical analysis of the aspirate for α-amylase activity was positive in all cases, which is a fundamental difference of this type of cyst.

In patients who underwent an MRI examination, the presence of tumor-like formations of various sizes with clear contours and reduced echogenicity of the contents was established in the lower parts of the gland (Fig. 2).

Operative intervention was carried out according to classical methods, while general anesthesia was used in 16 patients (76.2%), and 5 patients (23.8%) were operated on under local anesthesia. Wound healing occurred by primary tension. All cystic formations were sent for further morphological and immunohistochemical examination.

The morphological structure of the walls of the cyst of the parotid salivary gland consisted of a connective tissue membrane, an epithelial lining, which was represented by a multi-layered flattened epithelium with basal and spiny cells, in some places the phenomenon of parakeratosis and papillary growths were observed. Areas of round cell infiltration with lymphoid substance and glandular epithelium acini were located under the epithelium layer, which were separated by thickened connective tissue membranes. It should be noted that in some areas the epithelial lining was absent. When the material was stained and the PAS-reaction was

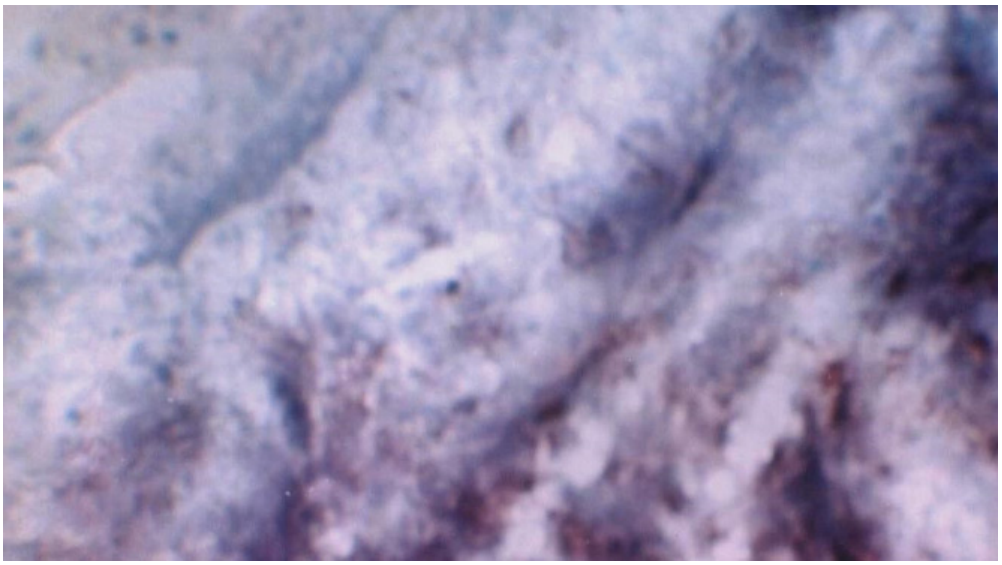

Fig. 6. Presence of CD8+ cells in the epithelial layer

performed, a positive result was established throughout the thickness of the epithelial layer (Fig. 3).

With the help of the performed immunohistochemical studies, it was possible to determine the presence of 2-3 HLA-DR+ cells per 100 epithelial cells, and a positive reaction in the form of a continuous band of HLA-DR+ inclusions was observed in the basal and subbasal layers, or moderate infiltration by them was observed within the acinar epithelium (Fig. 4).

In addition, CD3+ cells were determined in the epithelium in the number of 1 to 7 per 100 epitheliocytes and they were the most numerous, along with HLA-DR+ cells (Fig. 5). The presence of CD4+ cells along with CD20+ cells in the epithelium was not detected, unlike the subepithelial layer, where they occupied significant areas.

In turn, the infiltration of CD8+ cells of the epithelial layer was established in the amount from 1 to 7 per 100 epitheliocytes. A moderate number of them was also determined subepithelially, and they were single directly in the cyst wall (Fig. 6)

DISCUSSION

From a scientific point of view, further research on establishing the nature of the redistribution of immunocompetent cells in different layers of the parotid salivary gland cyst can clarify some aspects of the embryonic laying and indicate its dysontogenetic origin. Taking into account the fact that the facial area is formed from three germ layers, there is a favorable situation for the formation of benign formations in this anatomical area in the process of embryogenesis.

In all cases, the final diagnosis is determined by the morphological structure of the cyst, but this is not always a guarantee of accurate verification, therefore, in difficult and doubtful cases, there is an urgent need for additional involvement of the newest technologies, which include the immunohistochemical method. In different layers of the cystic membrane, certain regularities of their location are observed and directly in the epithelial layer they are represented by small numbers of HLA-DR+, CD3+ and CD8+ cells; the subepithelial layer is intensively infiltrated with HLA-DR+, CD3+ and, to a lesser extent, CD4+, CD8+, and CD20+; HLA-DR+, CD3+ and a small number of CD8+ cells are located around the acini. In the connective tissue of the capsule, immunocytes are not represented.

Shynkevych VI et al. (2021) also used an immunohistochemical method to determine the density of CD68+ and CD163+ cells as preliminary morphological equivalents of different subpopulations of Mφs. The molecules CD68 and CD163 are scavenger receptors that contribute to the polarization program of these cells. In turn, Avetikov DS et al. (2020) determined the influence of polymorphism of collagen type I alpha-2 gene (COL1A2) (rs42524) on the formation of scar tissue localized in the head and neck region. As a result of these studies, it became possible to indirectly indicate the activation of the protective reaction of the skin to physiological scarring and dosed formation of scars in different areas of the head and neck [16-18].

Immunohistochemical studies have proved to be particularly informative in hematological and oncological practice, thanks to which it became possible to verify rare tumors of various origins. Thus, the research of Magda Zanelli et al. (2021) found that immunohistochemical expression of LMO2 together with some morphological clues can help identify cases of T-LBL in the background of M/LNs-Eo, stating that this group

of disorders can be easily underdiagnosed due to both rarity and proteiform clinical presentation [19].

Andrew M Bellizzi, M.D. (2022) in his review "An Algorithmic Immunohistochemical Approach to Define Tumor Type and Assign Site of Origin" gave answers to a whole series of questions, but he paid special attention to the morphological and immunohistochemical study to determine special risk groups from the point of view of diagnostic considerations regarding keratin broad spectrum of action/CD45/S-100-"triple negative" neoplasm [20].

Unfortunately, there is too little information in the literary sources, which makes it impossible to carry out a detailed comparative comparison of the results we obtained. The general clinical characteristics of parotid salivary gland cysts given in our work do not differ from the existing classical ones, in contrast to the information obtained when applying additional and special research methods, especially regarding the establishment of features of redistribution of immunocompetent cells in different layers of the cystic membrane. This makes it possible to supplement scientific data on the role of immunocompetent structures in the formation of immunological potential, and, accordingly, on the strength of the immune response to external and internal factors at the level of organs, systems and pathological formations, which was the focus of attention in our previous publications [7,12].

CONCLUSIONS

Thus, our research proves the importance of the implementation of the latest technologies and their high informativeness when studying the immunocompetence of individual layers of the wall of parotid salivary gland cysts. Directly, the immunohistochemical method makes it possible to establish the type of redistribution of immune cells in them, which is very important when conducting differential diagnosis in difficult and doubtful cases.

In general, establishing the characteristics of the distribution of cells in tissues can serve as a highly informative test when specifying the nosological form of the disease in complex and doubtful cases, and can also be suitable for predicting the likelihood of an inflammatory component and the possibility of malignancy of certain structural elements of the cystic membrane.

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REVIEW ARTICLE

CONSIDERATIONS FOR INTRADERMAL APPLICATION OF IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES IN MEDICINE

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ABSTRACT

The aim: Analyzing literature sources, to assess possibilities of using the method of intradermal immunization with native autoleukocytes to treat different diseases, to investigate the areas of usage, efficacy and expediency of the technique in clinical practice.

Materials and methods: Analysis of literature sources associated with intradermal immunization with native autoleukocytes.

Conclusions: The possibilities of using the method of intradermal immunization with native autoleukocytes in the treatment of various diseases are considered in the literature review. Intradermal immunization with autoleukocytes is one of the methods of personalized medicine. The application of the method results in normalization of the immune system condition as well as suppression of autoimmune and inflammatory processes. It also reduces the synthesis of pro-inflammatory cytokines and strengthens cellular antiviral immunity in a number of viral infections. It is proved, in particular, that the method reduces the synthesis of cryoglobulins, the formation of antithyroid antibodies, normalizes the level of tumor necrosis factor alpha, as well as reduces extrahepatic manifestations of chronic hepatitis and increases the effectiveness of antiviral therapy in patients with viral hepatitis B. Considering that immunization with native autoleukocytes has no contraindications, it can be used in many diseases.

KEY WORDS: antithyroid antibodies, antiviral therapy, cryoglobulinemia, antinuclear antibodies, immunization with autoleukocytes, tumour necrosis factor α

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INTRODUCTION

For complex treatment of a wide range of diseases, medicine involves the methods, which belong to cellular technologies [1-5]. The latter usually encompass a set of techniques that imply isolation of certain cells from the body, their cultivation for multiplication or imposing of antigen-presenting properties, frequently stimulation for the synthesis of metabolic products, in particular interleukins, with subsequent use of these cells or their synthesized substances [1]. Currently, application of stem cells is considered the main and the most perspective direction of the cell therapy [6-8].

However, we suggest considering intradermal immunization with native autoleukocytes (IINAL), isolated from peripheral venous blood, as one of the effective directions in the cell therapy [2, 3]. IINAL is the method of a personified cell therapy, which is a perspective method of treating various diseases, particularly autoimmune ones. Presence of different transmission factors in lymphocytes makes this procedure (immunization) similar to a vaccine by its mechanism of action [1]. Besides, replication of viruses occurs in leukocytes in a number of viral infections, thus, they can be used as individual virus-containing material, which definitely distinguishes this method from standard therapeutic vaccines due to various mutations of a virus [4].

THE AIM

Analyzing literature sources pertaining to intradermal immunization with native autoleukocytes, to investigate the areas of usage, efficacy and expediency of the technique in clinical practice.

MATERIALS AND METHODS

Analysis of literature sources associated with intradermal immunization with native autoleukocytes.

REVIEW AND DISCUSSION

Peculiarities of the method of intradermal immunization with autoleukocytes: 1) isolation of leukocytes from heparinized venous blood by its precipitation

for a few hours at t +37°C; 2) intradermal injection of leukocytes into the back region (between scapulae) into 8-10 points. This technique is described in detail in medical literature [2-5].

At present, the efficacy of such procedure has been proven for the treatment of many diseases by stimulating (or normalizing) the immune system, in particular, elimination of allergic conditions, decrease in intensity of inflammatory processes, as well as an antiviral vaccine. Some variations of curative therapy by means of intradermal immunization with native autoleukocytes will be discussed in the article.

I. APPLICATION OF INTRADERMAL IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES IN THE TREATMENT OF AUTOIMMUNE PROCESSES

1. Impact of immunization with autoleukocytes on cryoglobulinemia syndrome.

Cryoglobulinemia syndrome is an immune-dependent condition caused by the presence of pathological proteins – cryoglobulins in the blood serum. They belong to immunoglobulins and can form insoluble complexes (cryoprecipitate) in temperature reduction below 37^0C [5].

Cryoglobulinemia syndrome is known to be characterized by a variety of clinical manifestations, which depend on the degree and localization of affected vessels. Clinical symptoms of cryoglobulinemia (inflammation and necrosis of the vascular wall) can be manifested by damage to the following organs (systems): skin and mucosa, bones and joints, muscles, blood, immune system, ear, nose and throat, lungs, heart and vessels, digestive organs, kidneys, nervous system, eyes etc. [5].

According to our findings, intradermal immunization with autoleukocytes can be efficiently used for the treatment of cryoglobulinemia. It has been established that even after one procedure, the level of cryoglobulins decreases by 40% and more in 82% of patients. In some cases, immunization should be performed several times for better treatment efficacy [6].

A wide range of pathological processes, caused by cryoglobulinemia, indicates that it is expedient to use IINAL in numerous diseases provoked by inflammation and necrosis of the vascular wall.

Thus, for instance, vascular pathology is known to be a common cause of spermatogenesis impairment. Thus, patients with impaired synthesis of spermatozoa were examined for cryoglobulins, and we studied possible association between cryoglobulinemia and idiopathic oligo- and asthenozoospermia. It was established in these investigations that over the third of men with idiopathic infertility had elevated level of cold-precipitating proteins in the blood – cryoglobulins of the second and the third types. Inhibition of cryoglobulin synthesis by the IINAL method had a positive impact on spermatogenesis in the majority of patients with idiopathic oligoasthenoteratozoospermia, and resulted in normalization of spermogram indices or improvement of conditions for conduction of assisted reproductive technologies for couples [7-10].

This technique is also used in chronic viral hepatitis, since numerous extrahepatic signs of chronic viral hepatitis (especially chronic hepatitis C) are due to cryoglobulinemia [11]. Thus, intradermal immunization with native autoleukocytes inhibits synthesis of cold-precipitating proteins and has a positive impact on extrahepatic signs of chronic viral hepatitis, particularly glomerulonephritis, arthralgia, skin vasculitis and other manifestations of an elevated level of cold-precipitating proteins [3, 11, 12].

Although efficient antiviral therapy in chronic hepatitis C promotes normalization of the immune functions, cessation (or reduction) of immunopathological processes, signs of cryoglobulinemia syndrome can remain in many patients after elimination of the virus. Thus, for example, fatigue was observed in some patients with chronic hepatitis C with general weakness (201 patient) after achievement of a stable virological response (in 107 individuals; 53.23%). Cryoglobulinemia was observed in most patients (93%) with fatigue after successful completion of antiviral therapy [13]. Thus, expediency of cryoglobulin synthesis cessation by means of immunization with autoleukocytes often appears even after recovery from chronic hepatitis C.

However, in chronic hepatitis B, unlike in chronic hepatitis C, vascular damage is usually caused by harmful action of circulating immune complexes, which contain virus antigens and antibodies. Nevertheless, a certain fraction of vasculitis can be referred to cryoglobulinemic ones. In our previous investigations on peculiarities of different forms of chronic hepatitis B in Western region of Ukraine, it was established that in most patients with HBeAg-negative HBV DNA-positive, systemic vasculitis developed along with cryoglobulinemia (in seven out of eight patients with vasculitis signs), whereas in the group of patients with HbeAg-positive hepatitis, it was observed only in one patient with vasculitis out of 10 [14]. Clinically, cryoglobulenemic syndrome in patients with HBV is most frequently manifested by vasculitis, acrocyanosis, polyarthralgia, restricted movements, slight signs of sensory polyneuropathy, kidney damage, increased sensitivity to cold. Thus, in case of such extrahepatic signs, intradermal immunization with autoleukocytes is also indicated [2, 3, 5].

It has been established that treatment of cryoglobulinemia by IINAL technique is also highly efficient for patients with chronic toxoplasmosis with impairment of hypothalamic region. Injection of own leukocytes to such patients resulted in a significant reduction of the level of cold-precipitating proteins in the blood, considerable improvement of patients' general condition, and in most cases it was characterized by a stable remission [15].

Thus, the reported data prove the expediency of inhibition of cryoglobulin synthesis by intradermal immunization, since the method is efficient and does not have contraindications.

2. Impact of immunization with autoleukocytes on antinuclear antibodies.

Detecting the level of antinuclear antibodies (ANA) is a laboratory test used for diagnosis of autoimmune diseases. ANA are present in the majority of patients with diffuse diseases of the connective tissue, can be detected in infectious diseases (especially chronic viral hepatitis) etc. After improvement of a patients' condition, ANA indices significantly decrease, even become normal. Thus, in the patients treated by means of immunization with autoleukocytes, ANA level was compared before and after the procedure as one of the methods for assessment of IINAL efficacy in patients with autoimmune processes, particularly in combination with HCV [3]. In a group of 20 patients, the initial ANA values were as follows: in 4 patients (20%) they were within the normal range (1:80), in 8 patients (40%) – 1:160, in 6 patients (30%) – 1:320 and 2 (5%) cases of antibody titers equaled 1:640 and 1:1280, respectively. Among patients with elevated ANA titers (16 people), the index decreased in almost all patients (15; 93.75%) in 28-72 hours after immunization with autoleukocytes. In 2 patients (12.5%), a 16-fold decrease was observed, in 4 - the titer fell 8-fold (25%), and in 9 (the largest number of observations; 56.25%) a 4-fold decrease in the level of antibodies was registered. Only in 1 patient (6.25%) with the highest ANA titer (1:1280), the level of antibodies remained at the previous level after immunization with autoleukocytes. It should be noted that as a result of a single intradermal immunization with native autoleukocytes, the ANA titer did not just decrease, but returned to the normal range in 14 out of 16 patients (87.5%) [2].

In other investigation of IINAL impact on ANA titres [11], the obtained results were similar, however, immunization with autoleukocytes proved ineffective in one patient, whose ANA titre increased from 1:80 to 1:1280 under the influence of antiviral therapy with pegylated interferon. After cessation of antiviral therapy, titre of antibodies spontaneously decreased to 1:640 and further remained at this level. However, even after single immunization, ANA were not detected already in 48 hours. Thus, this procedure also proved efficient in this observation.

3. Impact of immunization with autoleukocytes on recovery of tolerance to the thyroid antigens.

Data about IINAL impact on the synthesis of antibodies to the thyroid antigens, which was tested for the first time on patients with chronic hepatitis B and C, who often had thyroid peroxidase and thyroglobulin antibodies, were analyzed [16, 17].

Besides, 22 patients with laboratory signs of autoimmune thyroiditis were included in one of the studies on the renewal of tolerance to thyroid autoantigens. The levels of thyroid peroxidase antibodies (TPO) and thyroglobulin antibodies (TgAb) were assessed before and 10–12 days after a single autoleukocyte immunization. A decrease in the concentration of antibodies was detected in all immunized patients, and the group with a significant decrease in indices (by 50-100%) was the largest in number. This group in the study case of a decrease in the level of thyroid peroxidase antibodies included 8 patients (36.36%), and for thyroglobulin antibodies – 10 (45.45%) individuals. Some examined patients demonstrated a normalization of the indices: 6 cases (27.27%) in determining TPO level, and 3 cases (13.64%) in studying TgAb indicators [17].

Subsequently, decrease in the level of antibodies was significant in the majority of immunized individuals, however, these indices were individual, duration of the obtained results also differed. Thus, the number of procedures and period of recurrent immunization is determined individually for each case. However, a positive result of immunization with autoleukocytes in relation to the thyroid antigens has been confirmed [17]. It is of utmost importance as the risk of autoimmune thyroiditis development is reduced.

II. APPLICATION OF INTRADERMAL IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES FOR REDUCTION OF INFLAMMATORY PROCESS INTENSITY.

Currently, a strategy of inflammatory processes therapy involves inhibition of high level of pro-inflammatory cytokines, in particular tumor necrosis factor alpha (TNF-alpha) by means of medicines, which block the formation of this cytokine or inhibit proliferation of Th-1-lymphocytes, producing TNF.

The drugs acting on inhibition and blockage of biological activity of TNF-α are used for the treatment of immune-mediated diseases, such as rheumatoid arthritis,

inflammatory diseases of the intestines and psoriasis. Clinical efficacy of such drugs is confirmed, however, inhibition of cytokine synthesis by means of antibodies to certain determinants of immunocompetent cells has a negative impact on immunity condition. Prolonged use of TNF-α inhibitors increases susceptibility to infectious diseases (or exacerbation of the existing ones). Considering these adverse manifestations, it is obvious that such methods are unfavorable. Therefore, IINAL was tested for inhibition of excessive TNF-α synthesis [18]. It has been established that this method has a positive impact on reduction of inflammatory process, particularly in patients with psoriasis [19].

Since the level of pro-inflammatory cytokine became normal almost in all patients and their condition improved after the procedure, the decision was made to test this method for reduction of TNF-α synthesis in patients with HBV [20]. Reduction of TNF-α in blood serum was also recorded in examined patients due to immunization. Their overall condition improved, which was manifested by reduction of general weakness and clinical signs of extrahepatic signs: skin vasculitis and kidney damage.

Emphasis should be made on the group of patients with HCV, who had very low level of TNF-α in blood serum or it was not detected at all. In some of them (approximately 30%), moderate increase in cytokine level occurred, which also promoted improvement of their condition.

III. APPLICATION OF INTRADERMAL IMMUNIZATION WITH NATIVE AUTOLEUKOCYTES AS A THERAPEUTIC ANTIVIRAL VACCINE.

It is known that viruse reproduction of some infectious diseases occurs in polymorphonuclear leukocytes and monocytes. Thus, blood immune cells can be used as a vaccine, which contains "own" patient's virus [1]. For example, hematogenous route of transmission, caused by interaction with leukocytes plays a significant role in pathogenesis of herpes infection (substantial accumulations of herpes simplex virus type 1 and 2 are found in leukocytes). Therefore, autoleukocytes can be used as an antiviral vaccine for this disease. IINAL proved an effective vaccination for the treatment of patients with severe form of type 1 and 2 chronic herpes infection [4].

It was also determined that immunization with patient's own native leukocytes temporarily inhibits intensity of hepatitis B virus replication [21]. Thus, the technique for intensification of antiviral therapy for chronic hepatitis B was also elaborated [22, 23]. The investigation included patients, in whom DNA HBV replication remained despite prolonged treatment with tenofovir (at least two years). This group included patients, who demonstrated reduction of viral load to a certain degree after achievement of "individual effect" of antiviral therapy, and it remained stable throughout observation period (8 months). Thus, immunization was performed three times with an interval of 30-40 days along with tenofovir treatment. Inhibition of virus reproduction resumed due to conducted immunization. Besides, patients with high viral load demonstrated better response to such therapy.

Thus, based on a trial of this method in patients with type 1 and 2 chronic recurrent herpes and chronic hepatitis B, it is obvious that it should be used as an antiviral therapy in other viral infections as well, provided the virus (virus antigens) is present in blood lymphocytes.

Expediency of applying IINAL method in the treatment of a wide range of diseases has been proven. For instance, clinical examples of IINAL immunization for patients with chronic hepatitis B, C and autoimmune hepatitis are presented in one medical article [24]. The examples demonstrate that in patients with hepatitis, the level of cryoglobulins significantly decreases, synthesis of antinuclear antibodies and thyroid antibodies is inhibited, overall condition improves after such immunization.

Thus, a wide range of intradermal immunization with native autoleukocytes enables to use them in various diseases. According to our previous data, the procedure is also efficient in rheumatoid arthritis, Bekhterev's disease, early stages of osteoarthrosis, Raynaud's disease, pollinosis, bronchial asthma etc.

The mechanism of positive impact of immunization with autoleukocytes on a wide range of immunopathological processes requires further investigations. However, it has been proven that IINAL amplifies an immune response by activating congenital immunity factors, cross reactivity by means of partial identity of antigen structures, antiviral surveillance by regenerating cytotoxic lymphocytes etc. The process of correction of Jerne immune network – idiotypic-anti-idiotypic regulation of the immune response, activation of CD3+, CD8+, CD25+-lymphocytes, as well as CD3+, CD8+, CD28+-lymphocytes is also important along with blockage of Fc-receptors and glycoprotein, pectin receptors on B-lymphocytes [1, 2].

CONCLUSIONS

Intradermal immunization with autoleukocytes is one of the methods of personified medicine. This method significantly improves the results of treatment of many diseases, has a positive impact on autoimmune processes, decreasing synthesis of pro-inflammatory cytokines,

amplifying cell-mediated antiviral immunity in numerous viral infections. Considering that immunization with native autoleukocytes does not have contraindications, it is expedient to use this method in many diseases, in particular those, where cryoglobulins, factors of autoimmune aggression, high level of pro-inflammatory cytokines, and the presence of a virus in leukocytes play an important role in pathogenesis.

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REVIEW ARTICLE

GAZING INTO THE ABYSS: TOWARD A PHILOSOPHICAL UNDERSTANDING OF ANXIETY AND FEAR

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ABSTRACT

The aim: This paper deals with anxiety or fear-related disorders and philosophical interpretations of the phenomena of fear and anxiety.

Materials and methods: The authors used integrative anthropological approach, interpretive research paradigm, hermeneutical approach. The data collection was carried out using Scopus, Web of Science, PubMed, Google Scholar databases. Research papers were identified according to search terms: "anxiety or fear-related disorders", "fear", "anxiety", "phobia", "psychology", "psychotherapy", "mental health", "philosophy", "being-in-the-world", "human existence".

Conclusion: It is obvious that philosophical interpretations of the phenomena of fear and anxiety cannot be defined in clinical terms, especially given the fact that modern psychology, psychotherapy, and psychiatry are shifting towards evidence-based practices. Furthermore, there are significant differences in determining the causes that provoke mental health disorders (nature, nurture, their interaction or something else) and accordingly in choosing treatment methods. Apparently, the position of the researchers/practitioners will be determined by their understanding of the root cause. However, more and more researchers are coming to the conclusion that the contemporary world demonstrates the urgent need for an integral, holistic paradigm of a human as an undivided, alive and organic, ideal and material being that should be understood in the context, taking into account the socio-cultural, biological, narrative, self-relational dimensions and his/her ability to transcend them. That is why the most effective way to help people with anxiety or fear-related disorders is to develop "the optimal combination of social assistance, psychological therapy, transpersonal experience, and medical treatment. It should be based on a new transdisciplinary paradigm and implemented by an interdisciplinary team of specialists". In this process, a special role should be assigned to philosophy, which is able to expand horizons and find an approach to the core of a human being.

KEYWORDS: anxiety or fear-related disorders, fear, anxiety, phobia, mental health, philosophy, being-in-the-world, human existence

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INTRODUCTION

The treatment of anxiety or fear-related disorders is a large-scale problem. Its complexity is related to a variety of manifestations, a wide range of possible emotional reactions, multiply risk factors for the development of the disorders, high levels of comorbidity. Anxiety or fear-related disorders negatively affect both an individual and society causing significant deterioration in a person's mental and physical state, a decrease in productivity, as well as a disability. They can be a predictor of increased suicidal feelings, a risk factor for the further development of depressive disorder, obsessive-compulsive disorder, various types of addiction, etc. The ICD-11 defines anxiety or fear-related disorders as disorders which "are characterized by excessive fear and anxiety and related behavioral disturbances, with symptoms severe enough to result in significant distress or impairment in personal, family, social, educational, occupational, or other important areas of functioning" [1]. Anxiety or fear-related disorders include generalized anxiety disorder, panic disorder, agoraphobia, specific phobia, social anxiety disorder, separation anxiety disorder, selective mutism, other specific anxiety or fear-related disorders. Anxiety and fear-related disor-

ders share phenomenological similarities, for example, physiological indicators, such as an increased heart rate, excessive sweating, and hyperventilation, or behavioral actions, such as escape and avoidance.

Undoubtedly, every person feels fear, because fear is a natural human emotion that helps to prepare for danger and respond adequately. However, some fears are more pronounced and are manifested as phobias. The word "phobia" is a derivation of the Greek word φόβος, which means fear, aversion, or morbid fear. A phobia is a debilitating fear that develops when someone exaggerates danger of certain objects or situations. The phobias make life extremely difficult, and in severe cases they can affect a person's health, well-being, and the whole way of life. The complexity of the modern world causes new fears/phobias that may still go unnoticed or be misinterpreted. Thus, the problem of diagnosis, interpretation, and treatment of anxiety or fear-related disorders is becoming more and more acute, especially taking into account the multidimensionality of a human being. That is why, for a comprehensive understanding of the phenomena of fear and anxiety, it makes sense to involve the representatives of various academic disciplines, in particular, philosophers who have for centuries tried to unravel the riddle of fear and anxiety and find ways to alleviate or overcome them.

THE AIM

This paper deals with anxiety or fear-related disorders and philosophical interpretations of the phenomena of fear and anxiety.

MATERIALS AND METHODS

The authors used integrative anthropological approach, interpretive research paradigm, hermeneutical approach.

The data collection was carried out using Scopus, Web of Science, PubMed, Google Scholar databases. Research papers were identified according to search terms: "anxiety or fear-related disorders", "fear", "anxiety", "phobia", "psychology", "psychotherapy", "mental health", "philosophy", "being-in-the-world", "human existence".

REVIEW AND DISCUSSION

The ICD-11 does not distinguish between phobic anxiety disorders and other anxiety disorders. Agoraphobia is considered as a separate diagnostic unit, and the panic attack specifier is used in conditions in which panic attacks are the reaction to the influence of agoraphobic situations [2]. The ICD-11 also emphasizes a wide range of possible emotional responses.

Up to now, there are different explanations for the development of anxiety or fear-related disorders. These are psychological factors, such as negative affectivity, neuroticism, and anxiety sensitivity; social factors, such as psychotraumatic childhood experiences, lack of emotional warmth or hyperprotection, adverse social experiences [3; 4]. The individuals with social anxiety disorder are often prone to safety-seeking behaviors. They try to predict the results of their actions, possible losses, avoid unpleasant situations that creates the vicious circle of anxiety [5]. Such control and "inhibition" are one of the early indicators of social anxiety, which can later manifest itself in low extraversion and high neuroticism, as well as contribute to the development of maladaptive cognitive schemes [6].

The cognitive model is based on the informational model of the human psyche and the study of the patients' dysfunctional beliefs about themselves, the world, and people around them. Safety behaviors (from the patients' point of view) allow them to avoid anxious situations, but do not give them the chance to transform the "distorted world" of their perceptions [4; 5; 7].

Genetic and physiological factors can play a significant role in the development of the disorders. For example, it is highly likely that phobic disorders are genetically heterogeneous [8]. The researchers have focused their attention on the study of genetic polymorphisms responsible for the manifestation of phobic disorders. Hauner emphasizes: "Under normal circumstances, fear triggers a natural fight-or-flight response that allows animals to react quickly to threats in their environment. Irrational and excessive fear, however, is typically a maladaptive response. For fear to escalate to irrational levels, a combination of genetic and environmental factors is very likely at play. Estimates of genetic contributions to specific phobia range from roughly 25 to 65 percent, although we do not know which genes have a leading part. No specific phobia gene has been identified, and it is highly unlikely that a single gene is responsible. Rather variants in several genes may predispose an individual to developing a number of psychological symptoms and disorders, including specific phobia" [9]. Contemporary neurobiological studies indicate the involvement of the amygdala and hippocampal complex, superior temporal sulcus, prefrontal and parietal cortex, and dorsal striatum in the development of the problem. Changes in the amount of white matter in certain brain tracts, which are responsible for cognitive functions and affective regulation, are considered as biomarkers. However, most studies stress that the combination of genetic and environmental factors is responsible for the development of the disorders [10; 11].

One more possible reason for the development of phobic disorders is inflammation: high levels of inflammatory markers, including cytokines and C-reactive protein, are associated with activation of the stress response of central and peripheral immune systems and the release of cytokines [12]. Social anxiety is additionally characterized by the increased cortisol response and the lower testosterone level. These neuroendocrine changes are associated with a disruption in the functioning of connections between the amygdala and the prefrontal cortex [6].

Such a variety of the causes of anxiety or fear-related disorders requires different methods of treatment. Most cases are treatable, but there is no single treatment that works for all of them. In some cases, a combination of different treatments is recommended. Some studies confirm the effectiveness of both psychopharmacotherapy (selective serotonin reuptake inhibitors, benzodiazepine and nonbenzodiazepine anxiolytics, atypical antipsychotics, etc.) and psychotherapy [13; 14; 15]. The choice of the therapy strategy depends on clinical symptoms, the stage of treatment, personality characteristics, patient's attitudes and expectations, resources, and organizational capabilities. There are pros and cons to both psychopharmacological and psychotherapeutic treatments.

Psychopharmacotherapy can improve a patient's condition faster than psychotherapy, it also requires less effort and resources. However, drug discontinuation may cause rebound effects and withdrawal syndromes. Instead, psychotherapy shows better results and more stable remission. Psychotherapy is one of the most fruitful approaches to the treatment of anxiety or fear-related disorders. To deal with the problem, mental health professionals turn to exposure therapy, cognitive-behavioral therapy, group therapy, hypnotherapy, mindfulness meditation, diaphragmatic breathing, progressive muscle relaxation, etc. [16 - 23]. However, psychotherapy options are also limited. It is ineffective in the cases such as the fear of self-disclosure, the lack of motivation to change, the secondary gain, the interpersonal sensitivity level, the inability to attend sessions regularly, the reluctance to participate in group therapy, etc. [24, 25]. Anyway, in many cases, a combination of psychopharmacotherapy and psychotherapy is optimal.

Unfortunately, despite a fairly wide choice of treatment options for anxiety or fear-related disorders, humanity cannot get out of the trap of constant fears and anxieties; their number is steadily increasing, which forces scientists to explore more deeply their neurobiological, physiological, behavioral roots, as well as major distinctions or similarities between fear and anxiety. Daniel-Watanabe and Fletcher emphasize: "many anxiety disorders will involve both acute experiences of fear and more diffuse experiences of anxiety. For example, specific phobia, a primary fear disorder, will also involve experiences of anxiety concerning potentially encountering the feared stimulus. The distinction between the two emotions remains useful, but we should avoid treating models of a single emotion as sufficient models of a psychiatric disorder, especially when differentiating between fear and anxiety, as we should not expect them to occur entirely independently of one another" [26]. Analyzing their review, Domschke refers to Heidegger and his interpretation of fear and anxiety [27]. And indeed, for a deeper understanding of the nature of fear and anxiety, they must be analyzed from the standpoint of philosophy.

Aristotle defines fear as a state of mind: "Fear may be defined as a pain or disturbance due to a mental picture of some destructive or painful evil in the future. Of destructive or painful evils only; for there are some evils, e.g., wickedness or stupidity, the prospect of which does not frighten us: I mean only such as amount to great pains or losses. And even these only if they appear not remote but so near as to be imminent… fear is caused by whatever we feel has great power of destroying or of harming us in ways that tend to cause us great pain" [28]. The phenomenon of fear reflects the bursts of all possible affects of the human psyche.

Analyzing the origin and nature of emotions, Spinoza stated: "Hope is nothing but unsteady joy, arising from the image of a future or past thing about whose issue we are in doubt. Fear, on the other hand, is an unsteady sorrow, arising from the image of a doubtful thing. If the doubt be removed from these emotions, then hope and fear become confidence and despair, that is to say, joy or sorrow, arising from the image of a thing for which we have hoped or which we have feared" [29, p. 143]. For Descartes, fear and hope are complementary phenomena. Fear is the disposition of the soul, and it is the fear in the face of the unknown. The experience of fear generates surprise, which gives rise to the cognitive activity of an individual [30]. Thus, fear is not an absolutely negative phenomenon, but it can contribute to the human development.

Hegel's philosophy demonstrates the same attitude towards fear. Hegel interprets fear as the fear of the Absolute – a human being clearly sees his/her finite nature, his/her mortality. However, the philosopher stresses the positive aspects of this fear seeing it as the beginning of wisdom: "And here the first attitude toward the absolute object is that of fear; for individuality knows itself as in regard to the absolute object only as accidental, or as something which is transient and vanishing. But this standpoint of separation is not the true relation. On

the contrary, it is what knows itself to be a nullity, and, therefore, something which is to be done away with and absorbed; and its attitude is not merely a negative one, but is in itself, or implicitly, positive. The subject recognizes the absolute substance, in which it has to annul or lose itself, as being at the same time its essence, its substance, in which, therefore, self-consciousness is inherently contained. It is this unity, reconciliation, restoration of the subject and of its self-consciousness, the positive feeling of possessing a share in, of partaking in this Absolute, and making unity with it actually one's own – this abolition of the dualism, which constitutes the sphere of worship" [31].

Kierkegaard was the first philosopher to distinguish between fear (Frygt), that has a specific cause, and painful, sucking fear-vertigo (Angest), a fear of Nothing devoid of rational explanations. For Kierkegaard, fear is the definition of the dreaming spirit, and as such it belongs to the realm of psychology. True fear is quite different from specific fears: in contrast to them, this fear is the reality of freedom as a possibility for a possibility. Such a fear is the prerogative of a human, because human beings, unlike animals, have a spirit. This true fear cannot be reduced to the feelings of worry, nervousness, or unease. It is closely connected with the pinnacle of temporary tension, the moment when we have to act in order to shape or reshape our destiny and our Self [32].

In the 20th century, the phenomenon of fear acquired the ontological status. A human being, as a thinking being, realized his/her finiteness in all its depth and tragedy, experiencing fear of life in this worldly existence [33]. Existentialist philosophers have often used the metaphor of the abyss: true fear is like vertigo when we gaze into the abyss. For Jaspers, fear is vertigo, but its power paves the way to absolute consciousness, and fear leads us to fearlessness which is Truth [34].

In Heidegger's philosophy, "Dasein" is "being-in-the-world" into which an individual is "thrown" by the very fact of his/her birth. Thus, all the tragic adventures of our consciousness start here when we are faced with existence and its finitude. According to Heidegger, the phenomenon of fear as a mode of state of mind may be considered from the three points of view, namely "(1) that in the face of which we fear, (2) fearing, and (3) that about which we fear" [33]. This fear is connected to something in this world: "the 'fearsome', is in every case something which we encounter within-the-world and which may have either readiness-to-hand, presence-at-hand, or Dasein-with as its kind of Being… That which fear fears about is that very entity which is afraid – Dasein. Only an entity for which in its Being this very Being is an issue, can be afraid. Fearing discloses this entity as endangered and abandoned to itself. Fear always reveals Dasein in the Being of its 'there', even if it does so in varying degrees of explicitness" [33].

Heidegger made an attempt to distinguish between fear and anxiety. He explained anxiety as the state in which the threat is nowhere: "Anxiety 'does not know' what that in the face of which it is anxious is. 'Nowhere', however, does not signify nothing: this is where any region lies, and there too lies any disclosedness of the world for essentially spatial Being-in. Therefore that which threatens cannot bring itself close from a definite direction within what is close by; it is already 'there', and yet nowhere; it is so close that it is oppressive and stifles one's breath, and yet it is nowhere" [33]. However, as the philosopher emphasizes, there is an essential connection between fear and anxiety: "And only because anxiety is always latent in Being-in-the-world, can such Being-in-the-world, as Being which is alongside the 'world' and which is concernful in its state-of-mind, ever be afraid. Fear is anxiety, fallen into the 'world', inauthentic, and, as such, hidden from itself" [33]. Thus, fear and anxiety are distinct but inseparable. They are immanent to each other and to life itself. These ontological phenomena are modes of human existence.

Sartre considered anxiety and fear to be integral parts of our being in the world. Being abandoned in the world, an individual does not have a certain future, so he/she is forced to choose his/her own life and Self. Therefore, abandonment is associated with responsibility and fear. According to Sartre, pure existence itself is disgusting and fearsome [35]. Fear is strong, deep, and specific. Anxiety cannot be expressed/defined so clearly; it could be felt when individuals are afraid of themselves. "The whole universe is pierced with anxiety" [36] and our freedom comprehends itself through anxiety. Any human being is his/her own project. In a word, we are our own Future, but there is a possibility not to become this Future. Thus, the meaning of our existence is always problematic that entails anxiety.

Both fear and anxiety can be defined and interpreted through the lens of our "presence in the world", our essential connections with the world itself, with other people and, ultimately, with ourselves. Accordingly, these experiences require explanations from many standpoints, taking into consideration the multifaceted nature of a human being. Therefore, we cannot but agree with Sartre that "we must refer to being-in-the-world and to being-for-others" [36].

Certainly, the representatives of medical, psychological, biological sciences, even philosophers, especially positivist ones, may be critical of this stance. For example, Tucke in the thesis "The Pathology and Etiology of

Philosophy" qualified philosophy itself and the deepest philosophical questions as anxiety, stating that anxiety "is a result of philosophical questions" [37]. Indeed, anxiety can be associated with an attempt to address these questions. However, the exit is usually where the entrance was. The willingness and ability to ask questions is the first step to solving a problem: "a journey of a thousand miles starts under one's feet" [38, chapter 64]. Ultimately, the world's philosophical thought has provided humanity with various ways to deal with fears and anxieties, including one of the basic fears – the fear of death. In our opinion, rather anxiety and fears make us search for answers to the basic questions of being in order to restore our ontological security.

CONCLUSION

It is obvious that philosophical interpretations of the phenomena of fear and anxiety cannot be defined in clinical terms, especially given the fact that modern psychology, psychotherapy, and psychiatry are shifting towards evidence-based practices. Furthermore, there are significant differences in determining the causes that provoke mental health disorders (nature, nurture, their interaction or something else) and accordingly in choosing treatment methods. Apparently, the position of the researchers/practitioners will be determined by their understanding of the root cause. However, more and more researchers are coming to the conclusion that the contemporary world demonstrates the urgent need for an integral, holistic paradigm of a human as an undivided, alive and organic, ideal and material being that should be understood in the context, taking into account the socio-cultural, biological, narrative, self-relational dimensions and his/her ability to transcend them [39-41]. That is why the most effective way to help people with anxiety or fear-related disorders is to develop "the optimal combination of social assistance, psychological therapy, transpersonal experience, and medical treatment. It should be based on a new transdisciplinary paradigm and implemented by an interdisciplinary team of specialists" [40]. In this process, a special role should be assigned to philosophy, which is able to expand horizons and find an approach to the core of a human being. As Daniel-Watanabe and Fletcher rightly point out, it is necessary to "consider the importance of other aspects of the experience of anxiety, such as uncertainty and avoidance" [26]. Definitely, uncertainty causes ontological dissonance [42, 43]. Desperately trying to struggle with uncertainty, people are sinking deeper and deeper into the abyss of fears and anxiety. It is at this point that philosophy can come to the rescue, philosophy that knows how to deal with uncertainty and chaos and provides a beacon of hope in the ocean of uncertainty. The 21st century is the time for philosophy to return to the throne and feel proud to carry out the great mission of the mother of all sciences.

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REVIEW ARTICLE

THE RESULTS OF PSYCHOLOGICAL READINESS FOR STUDYING IN MEDICAL STUDENTS

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ABSTRACT

The aim: To analyze the psychological readiness of the first-year students to study at a medical university, difficulties in the educational process, and the formation of positive motivation to educational and professional activity.

Materials and methods: In the course of research, the following techniques have been used: bibliosemantic method for the analysis of scientific publications, methodology for study the main motives for choosing a profession, suggested by S. Yu. Daukilas, methodology for study the motives of academic activity, designed at the Department of Ukrainian Studies and Humanitarian Training of Poltava State Medical University (modified by A. Rean, V. Yakunin) to conduct a survey in the process of learning Ukrainian as a foreign language by the students of Medical Faculty at the Poltava State Medical University in 2021/2022 academic year.

Conclusions: Professional identity of medical students begins at the first course of study. First-year students' faces heavy academic load of general scientific, medical and biological disciplines, foreign languages, etc. It is important from the first days to create a positive psychological motivation for studying, for acquiring knowledge and skills that will be required in future professional activities. Therefore, the adaptation of educational material to the new student reality, the search for relevant forms of education, considering the individual psychological characteristics and cognitive capabilities of the new generation, the use of modern specialized multimedia tools, the involvement of students in independent research activities, etc. is of decisive importance for the formation of students' readiness for education in medical university. The study showed that the students were motivated to choose their medical profession with the method of self-reproducing for the first time and later the strength of this motive grew. The role of some motivational factors in the field of professional life, such as a personal pattern, personal life, and self-satisfaction were not so well formed. We also fixed a great motivation to implement professional knowledge abroad. Prospects for further research encompass the study of personal characteristics affecting the professional activity of future doctors is expected, including their psychological adequacy to the requirements of the profession - character, temperament, intelligence, communicative and organizational abilities, etc.

KEY WORDS: first-year students' psychological adaptation, psychological characteristics of the new generation, readiness for professional activity, motivation, medical students, educational and professional activity of students

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INTRODUCTION

Preparation of medical students for professional activities has been the object of scientific works by domestic and foreign researchers on more than one occasion. This issue was considered from the point of view of its structure, stages of formation, etc. In our opinion, psychological characteristics of the first-year students, issues of educational motivation and difficulties in the educational process generally remain unresolved. The changes that are taking place in the world actualize the need to conduct this research, which will contribute to improving the professional training of future doctors.

THE AIM

The aim of the research is to identify the main motives for choosing the physician's profession; provide practical recommendations for the formation of positive motivation for learning, as well as professional and cognitive interest.

MATERIALS AND METHODS

A socio-psychological study of the first-year students' readiness for a future profession and motives for choosing a profession was conducted according to the methodology proposed by S. Yu. Daukilas, methodology for study the motives of academic activity, designed at the Department of Ukrainian Studies and Humanitarian Training of Poltava State Medical University (modified by A. Rean, V. Yakunin) to conduct a survey in the process of learning Ukrainian as a foreign language by the students of Medical Faculty at the Poltava State Medical University in 2021/2022 academic year. We conducted a

survey by the students of Medical Faculties at the Poltava State Medical University in 2021/2022 academic year.

REVIEW AND DISCUSSION

The effectiveness of performing any type of activity largely depends on the formation of certain psychological qualities in the individual, which, in particular, includes psychological readiness and motivation to study.

O. Tarnovska proposed to understand psychological readiness for activity as an integrative formation consisting of three mutually determined and interdependent substructures: 1) functional, which includes components: motivational (motives for choosing a profession and specialty, orientation to its values, etc.); cognitive (knowledge of special disciplines); operational (availability of skills and abilities necessary for solving professional tasks, modulating one's activity); 2) emotional (disposition to solve professional tasks, self-confidence as a specialist, state of satisfaction with the profession, etc.); 3) personal (professionally important personality qualities: strong-willed, moral, etc.) [1].

E. Dyachenko and L. Kandybovich attributed the following to the components of psychological readiness:

1) motivational (positive attitude to the profession, interest in it and other sufficiently stable professional motives);

2) orientational (knowledge and understanding the features and conditions of professional activity, its requirements for the individual);

3) operational (possession of methods and techniques of professional activity, necessary knowledge, skills, abilities, processes of analysis, synthesis, comparison, generalization, etc.);

4) volitional (self-control, the ability to manage the actions that make up the performance of work duties);

5) evaluative (self-assessment of one's professional preparation and compliance of the process of solving professional tasks with optimal work samples) [2].

All five of the above components can be considered as the perspective of long-term readiness, and the first, fourth and fifth as the perspective of short-term readiness.

The future doctor's readiness for professional activity takes shape even before the start of professional training at a medical institution of higher education. N. Smila proved that readiness for professional activity is a complex formation that reflects the level of development of professionally important qualities and abilities of a specialist [3].

The author emphasizes that the active stage in the formation of psychological readiness includes the following stages: choosing a profession, enrolling in training, adapting to training and, ultimately, to professional activity.

Classifications very close to the above are given in the works of V. Bochelyuk [4], L. Matohnyuk [5], O. Cherepyekhina [6] and M. Antipov [7].

Scientists claim that the first year of study at a medical institution of higher education is the stage of "initial education". At this stage, first-year students have a noticeable decrease in attention, cognition, communication and their organizational abilities. At the same time, first-year students need to learn to master professional standards and improve communication. Students master the rules and regulations of the profession, begin to qualify themselves professionally. At this stage, students need to learn a lot of new information. It is obvious that at this stage students' professional development begins and the educational load of the disciplines of the cycle of general training, the cycle of professional training, and the cycle of practical training increases significantly.

Therefore, readiness for professional activity is a complex dynamic structure containing the beliefs of an individual, his views, motives, feelings, volitional and intellectual qualities, knowledge, skills, and abilities. Such readiness is achieved in the process of moral, psychological and professional training and is the result of versatile development of the personality taking into account the requirements determined by the specifics of professional activity [8].

While studying at a medical educational institution, students must master modern knowledge of elementary subjects and show the ability to apply the acquired knowledge in practical situations, collect information, determine its level of reliability and critically evaluate it, use information obtained during training and self-improvement [9]. At the initial stage of education, students begin to form a professional identity that corresponds to the third stage of professional development. Therefore, we consider it expedient to consider in detail the motivational component of the readiness of students of a medical educational institution for professional activity.

Education seekers often use the following groups of motives on the way to choosing a profession: choosing a profession «for the company»; interest only in the external side of the profession; «black and white» version of ideas about the essence of professional activity; pressure from parents or other adults; profession for prestige; attitude towards the person representing the profession and attitude towards the profession itself.

A socio-psychological study of first-year students' readiness for a future profession and motives for choosing a profession was conducted according to the methodology proposed by S. Yu. Daukilas, A. Dumchene [10]. Respondents rated the degree of their attitude

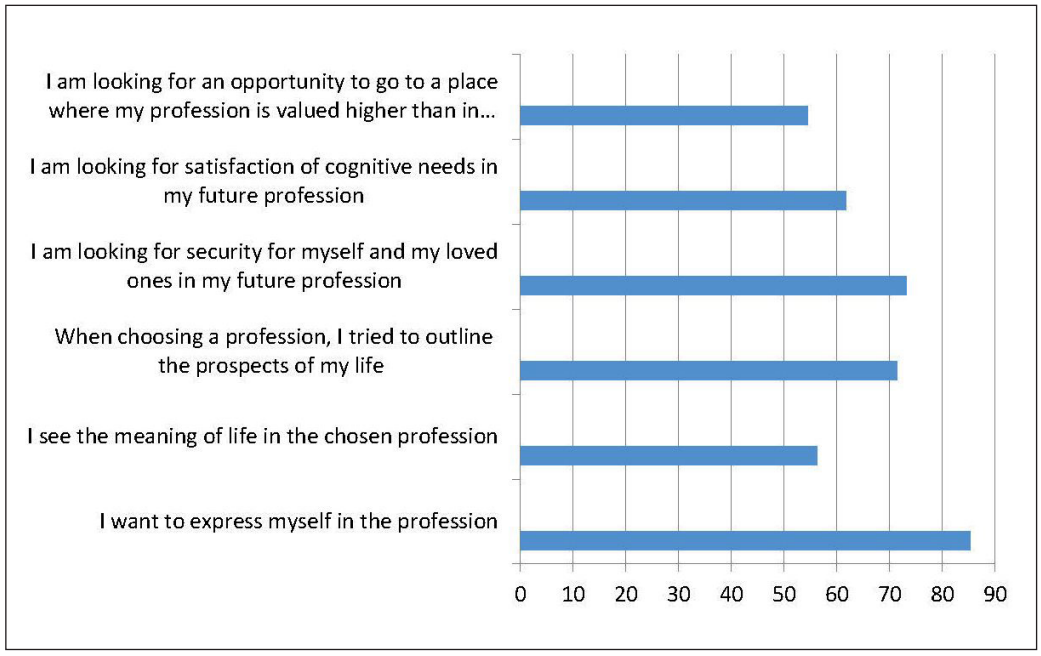

Fig. 1. The main factors that determined the choice of profession

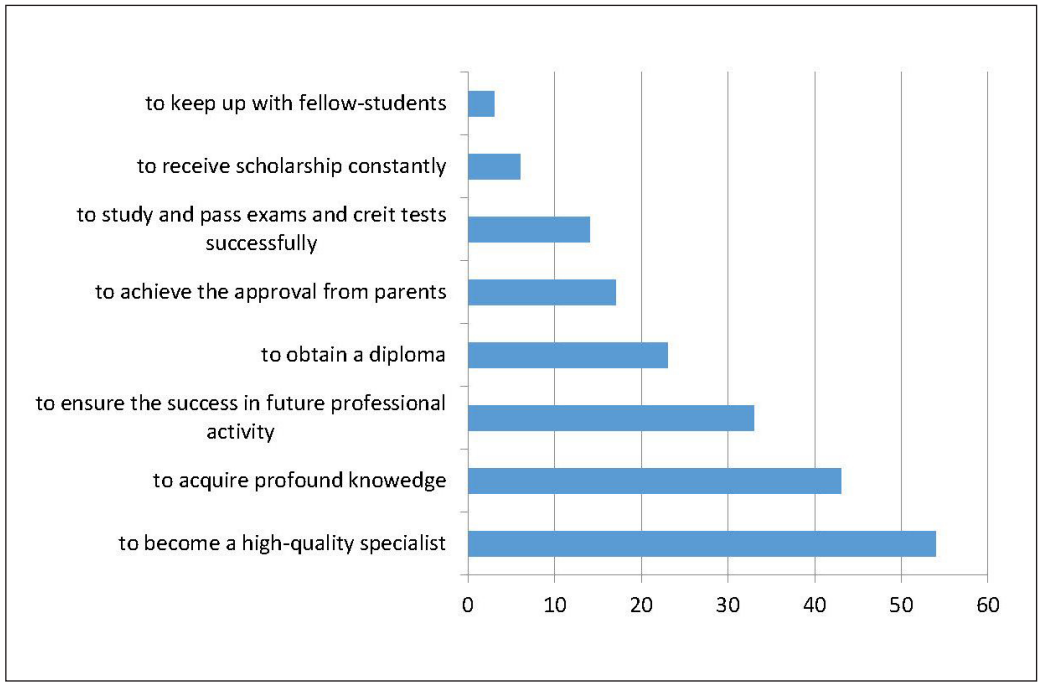

Fig. 2. Distribution of the results of the study in terms of motives of educational activity by the modified method of A.A. Rean and V.A. Yakunin

towards the provisions stated in the questionnaire as: agree, neutral, disagree.

We analyzed the learning process of 100 first-year students (medical faculty of PDMU) at the Department of Ukrainian Studies and Humanitarian Training during the study of the Ukrainian language in a professional direction. The peculiarity of the department of Ukrainian studies and humanities training is that its subjects are among the first to form the professional competence of the future doctor, they ensure the implementation of the educational program of the basic level of higher education in the professional direction of the disciplines assigned to the department.

The results of the study allow us to state that the majority of respondents, according to their personal traits and professionally important qualities, chose a profession according to the "Human - Human" type, whose representatives are characterized by: patience and demandingness, the ability to take responsibility, empathize and help, sensitivity, endurance, skill to restrain emotions, the ability to understand people, understand relationships, the ability to settle differences between them, organize their interaction.

The analysis of respondents' answers shows that 90% of future doctors have a pronounced orientation towards professional activity in working with people

Table I. Attitude of first-year students towards the object of their chosen profession

№	Statement	Agree	I am neutral	Disagree
1	In my profession, I would like to work with people.	90	7	3
2	I believe that I am able to resolve conflict situations.	65	20	15
3	I believe that I could patiently, without irritation, explain to other people what they do not understand.	73	15	12
4	I believe that I have tendencies towards artistic creativity and etc.	54	10	36
5	I want to engage in organizational activities.	41	35	24
6	I would like to conduct research that requires precision and accuracy	56	28	16

(table I), and only 3% of first-year students do not demonstrate such an orientation. At the same time, 65 students want and think that they know how to solve conflict situations. Among those who do not agree with the statement "I believe that I know how to solve conflict situations," 15% of first-year students. The majority of students, 73%, believe that they would be able to patiently, without irritation, explain to other people what they do not understand. A much smaller proportion of students (41% of first-year students in total) would like to be involved in organizational activities.

A significant proportion of students - 56% of first-year students demonstrate a tendency to clearly fulfill their duties ("I would like to conduct research that requires accuracy and accuracy"), which is in great demand for medical professionals. Such data show that students make the right choice of profession. Some of the students demonstrate personal qualities that are characteristic of other types of professions, along with the propensity for professional activity to work with people. 54% of first-year students demonstrated the possibility to engage in professions whose subject of work is the creation of artistic objects, choosing the statement "I believe that I have inclinations towards artistic creativity, etc."

The analysis of respondents' answers to the question about the motives for choosing a profession (Fig 1) showed that a significant majority of students (85%) choose the profession of a medical worker consciously in order to express themselves in the profession. More than 57.1% of students see their future profession as their life's purpose. When choosing a profession, 71.2% of future doctors sought to shape their life prospects. 73.4% of first-year students sought security for themselves and their loved ones in their future profession, 61.2% of students sought satisfaction of cognitive needs. Unfortunately, a rather significant share of students (54.3%) saw the advantages of their future specialty in the fact that it gives more opportunities to go to work in other countries, where the medical profession is valued more than in Ukraine.

In the course of the study, along with the motives for choosing a profession, we investigated the motives of educational activity by the modified method of A.A. Rean and V.A. Yakunin. We interviewed 100 first-year students (medical faculty of Poltava State Medical University) at the Department of Ukrainian Studies and Humanitarian Training while studying Ukrainian. Having evaluated the results, we found the following distribution of motives (Fig. 2).

The test results indicate that there is a lack of readiness for professional activity and motivation for learning. In our opinion, the formation of readiness for professional activity is influenced not only by the content of educational material, but also by other factors: the forms and methods of teaching, relationships between participants of the educational process, the psychological atmosphere during classes, etc.

In our opinion, development of positive motivation for educational activity is of great importance for the formation of readiness for future professional activity. For holistic formation of motivation for educational activity in future physicians, the formation process should be systematic. An important element in the development of motivation is the interest, which is divided by the scientists into emotional, intellectual, professional and cognitive.

Emotional interest arises when special attention is drawn to what evokes positive emotions and is pleasant. Intellectual interest is associated with knowledge of the world and intellectual activity of a man. Professional and cognitive interest is defined as the integrated formation of the individual, which is expressed in the constant desire to understand new knowledge in the future profession and as a form of expression of cognitive needs that provide the professional orientation of the individual. Therefore, the essence of professional and cognitive interest is to independently acquire the necessary knowledge and use it to solve professionally important tasks.

In our opinion, the formation of professional and cognitive interest in education is due to the diversification of educational activities, involvement of future specialists in the self-directed research activities, and solving tasks of the professional nature. For

Table II. Educational Apps Designed for Learning Ukrainian Language

Application name	System / OS	Functions	QR-Code
Ukraine Podcasts	iOS / Android	It is a series of podcasts for students learning English, which can be downloaded to mobile phones and engaged in any time convenient for the user. Each issue lasts approximately 20 minutes and is accompanied by questions for listeners.	
Learn Ukrainian Words Free	iOS / Android	It is a vocabulary trainer for learning Ukrainian that contains flashcard dictionary with Ukrainian-English translations of 10,000 words carefully organized by topics, for instance Travel, Business, Dating, Study or School. The mobile application also includes 100% Free Ukrainian lessons for beginners, advanced learners, adults and kids.	
Duolingo	iOS / Android	The educational course is divided into stages according to the principle «from simple to complex». The Duolingo educational application boosts such skills as writing and speaking (you will be asked to pronounce the studied phrases), reading and listening.	
Ukraine Podcasts	iOS / Android	It is a series of lessons that involves a rich mix of listening and reading activities, so student can make sense of written and spoken contexts. A simplified learning environment presents content structure, review progress, and learning activities front and center, while streamlined navigation encourages students to explore different topics in their target language.	
Simply Learn Ukrainian – Ukrainian Travel Phrasebook	iOS / Android	App is a free language app that will assist you to speak Ukrainian quickly and effectively when visiting Ukraine. Its content includes basic conversational phrases and topical vocabulary. Educational data is divided by levels of education background. It also provides questions, tests, or exercises to monitor closely students' learning process.	
Clozemaster	iOS	Clozemaster is gamified language learning through mass exposure to vocabulary in context. It contains listening transcribe mode punctuation fix, single and double quote fix, new SRS controls with legacy controls toggle, days played calendar with daily stats table.	
Learn Ukrainian. Speak Ukrainian Bluebird Languages	iOS / Android	App provides free daily lessons for beginner, intermediate and advanced learners. It contains interactive video lessons (over 5 years of lessons) that are aimed at improving speaking and understanding Ukrainian.	

instance, in teaching when learning the Ukrainian language, we recommend using the following online resources. Open access offers mobile applications of varying degrees of complexity (FunEasyLearn (https://play.google.com/store/apps/details?id=com.funeasylearn.ukrainian&hl=uk&gl=US), Language Course S.L (https:// play.google.com/store/apps/details?id=net.languagecourse.vt.uk&hl=uk&gl=US), Learning and playing Ukrainian language (https://play.google.com/store/apps/details? id=free.langame_ua.rivex&hl=uk&gl=US), Simply learn Ukrainian (https://play.google.com/store/apps/details?id=simply.learn.ukrainian&hl=uk&gl=US), Mondly: Learn Ukrainian Easily (https://play.google.com/store/apps/details?id=com.atistudios.mondly.ua&hl=uk&gl=US), Learn Ukrainian – 50 languages (https://play.

google.com/store /apps/details?id=com.goethe.uk&hl=uk&gl=US), Tobo Learn Ukrainian dictionary (https://play.google.com/store/apps/details?id=free.langame_ua.rivex&hl=uk&gl=US), LuvLingua (https://play.google.com/store/apps/details?id=com.luvlingua.learnukrainian&hl=uk&gl=US), LingQ (https://www.lingq.com/ru/learn/uk) etc.

The use of these resources makes it possible to expand the field of opportunities for the formation of learning motivation through new types of activities and new opportunities for realizing one's own competencies. The need to use the above-mentioned resources is due to the need to find information for practical classes, work on individual tasks, and independent work.

We paid special attention on to the didactic performance of educational mobile applications and their capabilities. This data is expressed in Table II. Applications from this list are suitable for teaching students with different levels.

An important aspect of students' educational activities in the context of studying a particular discipline is the individual work with educational material. Thus, students independently search for the necessary information, systematize the obtained search results, illustrate the submitted material with images from scientific articles, report on the results of the work, explaining the essence of the phenomenon based on the example of its clinical use [11].

CONCLUSIONS

A clearly expressed orientation of first-year medical school students to professional activities related to working with people (90% of research participants) was revealed, which indicates the correspondence of psychological and professionally important qualities to the chosen profession.

It was established that the students chose the medical profession motivated, in order to express them in it. However, the role of individual motivational factors in the choice of a profession, such as the vision of the meaning of life in the future profession, satisfaction of one's cognitive needs, must be developed in the learning process.

During the first year of study at higher education medical institution, students just begin to identify themselves with future professional activity, they learn the rules and norms of the profession, they are exposed to a large educational load, decrease their will, cognitive and communication skills.

During this period that it is very important to develop positive motivation for learning, acquiring knowledge and skills that will be needed in future professional activities. We suggest using modern multimedia tools (electronic journals, specialized websites) to form positive motivation for studying in the first year of medical faculty students; involve students in independent research activities, etc.

It is necessary to adapt the educational material to the new student reality, to search for relevant forms of education, considering the individual psychological characteristics and cognitive capabilities of the new generation.

We can motivate the students to educational and professional activities which are manifested in the awareness of the subjects of actual needs, such as education, self-development, professional development and others, satisfied through the implementation of educational tasks and encouraging them to study academic disciplines and master the skills of future professional activities. We consider the following pedagogical conditions effective for the formation of positive motivation of study in the first-year medical faculty students: to win modern multimedia research (electronic journals, specialized sites); get students to independent research activities and so on. It is necessary to adapt the educational material to the new student reality, to search for relevant forms of education, considering the individual psychological characteristics and cognitive capabilities of the new generation.

Thus, in modern conditions, the role of psychological and pedagogical support of the learning process is extremely increasing. Prospects for further research is to study of personal characteristics affecting the professional activity of future doctors is expected, including their psychological adequacy to the requirements of the profession - character, temperament, intelligence, communicative and organizational abilities, etc.

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REVIEW ARTICLE

MEDICAL USE OF VEGETABLES IN THE PRACTICE OF ANCIENT DOCTORS (BASED ON THE TREATISE OF QUINTUS GARGILIUS MARTIAL *MEDICINAE EX OLERIBUS ET POMIS*)

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ABSTRACT

The aim: The aim of our study is to investigate the specifics of the use of vegetables for the prevention and treatment of diseases in the medical practice of the Roman Empire.

Materials and methods: The research material was based on the surviving fragments of the Roman writer Quintus Gargilius Martial's *Medicinae ex oleribus et pomis*. The study relied on general scientific methods of analysis and synthesis, as well as the method of contextual analysis, descriptive and interdisciplinary methods.

Conclusions: Gargilius' treatise *Medicinae ex oleribus et pomis* is a valuable source of information on the use of vegetables, herbs, fruits, and nuts for the treatment and prevention of numerous diseases. Roman doctors highly valued the therapeutic and prophylactic properties of radish, pumpkin, cucumber, celery, beetroot, cabbage, turnip, rutabaga, lettuce, onion, garlic and other vegetables, and successfully used vegetables as a medicine to strengthen the immune system, enrich the body with vitamins and minerals, as well as for the prevention and treatment of wounds and injuries in surgery, various tumors and inflammations, diseases of the gastrointestinal tract, gynecological pathologies, fever, cough, diseases of the ENT organs and skin. For medicinal purposes, the Romans used leaves, stems, roots and seeds of various vegetable crops. The results of the study suggest the possibility of exploiting the therapeutic potential of vegetables in modern medicine.

KEY WORDS: history of medicine, Gargilius, vegetables, treatment, complimentary alternative medicine

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INTRODUCTION

Already in ancient times, people realized that vegetables, herbs and fruits are not only food that the human body should receive every day, but also a natural source of natural vitamins and essential minerals and nutrients. Eating vegetables and fruits increases the secretion of digestive glands, their enzymatic activity, and promotes better digestion of food. In addition, some vegetables contain phytoncides, which have a beneficial effect on the body. These properties of vegetables, fruits, and berries make them essential in a person's daily diet and play a significant role in the formation of immunity. In this aspect, it is important not only to refer to Latin medical texts and the experience of ancient physicians, but also to analyze the adaptation of their contribution to the history of medicine and pharmacology.

THE AIM

The aim of our study is to investigate the specifics of the use of vegetables for the prevention and treatment of diseases in the medical practice of the Roman Empire.

MATERIALS AND METHODS

The research material was based on the surviving fragments of the work of the Roman writer Quintus Gargilius Martial, known in the history of medicine as Book IV of the Medicina Plinii entitled *Medicinae ex oleribus et pomis*. (Medicines from Vegetables and Fruits) [1]. The work contains a description of the medicinal properties of 60 herbs, vegetables and fruits and in fact is the only document that attests to the existence of Roman medical literature in the third century CE.

The defining feature of the methodological basis of the history of medicine is the use of a combination of general scientific and special methods. The method of contextual analysis allows identifying and studying textual fragments, and analyzing the beneficial and medicinal properties of various vegetables. The descriptive method is necessary for the accumulation of information and further theoretical comprehension of the medicinal use of vegetables in the practice of ancient physicians. The interdisciplinary method made it possible to comprehend the interaction of the history of medicine with the basics of natural sciences and pharmacology.

REVIEW AND DISCUSSION

A review of the scientific literature shows that the study of the creative legacy of Quintus Gargilius Martial has been the subject of research by historians and philologists. The analysis has revealed that modern researchers have mostly translated Gargilius' *Medicinae ex oleribus et pomis* into modern languages. For example, the English translation of the work is presented in Root Tapper's doctoral dissertation [2]. In Joon Riddle's research, one can find fragments of the English translation of Gargilius' treatise and a characterization of his work as an important source of knowledge in the field of pharmacology and nutrition [3]. The issue of researching Gargilius' legacy is thoroughly explored in the investigations of Maire Brigitte. For instance, in the article 'L'esprit ou la lettre: les sources des Medicinae de Gargilius Martialis,' the researcher analyzes the question of Gargilius' medical knowledge origins and sources [4]. One of Maire Brigitte's contributions is her adaptation of approximately 60 chapters of the poem into French for contemporary readers whose interests lie in plants, their history, and their usage in ancient times [5]. The importance of Gargilius for the dissemination of knowledge about the healing properties of cultivated plants is also mentioned in the collective monograph 'Greek' and 'Roman' in Latin Medical Texts. Studies in Cultural Change and Exchange in Ancient Medicine [6].

The issue of reception of medical advice on the use of herbs, vegetables, and fruits as a means of alleviating or curing a disease, proper nutrition, and maintaining health based on Gargilius' work is presented in the study by Brodersen Kai [7]. The use of thyme as a snake repellent according to Gargilius' poem is partially discussed in the article by Arsenio Ferraces Rodríguez [8]. The attention of researchers of Roman literature of the late empire and historians of medicine is drawn to the translation of Gargilius' treatise into Polish by Tatiana Krynicka (Krynicka). T. Krynicka's work is not only the second (after the French) complete translation of the ancient author's work into modern languages, but also an encyclopedia on the prevention and treatment of various injuries and diseases with the gifts of nature. Furthermore, in the preface, the researcher thoroughly analyzes the life and work of Gargilius, his knowledge of ancient medicine, and the bibliography of his work, which allows her to draw a conclusion about the author's critical and polemical attitude to source texts [9]. In the context of our study, it is also worth noting a thorough review of the translation of Gargilius' treatise into Polish by Magdalena Stuligrosz [10]. Selected recipes for using vegetables and fruits based on Gargilius' treatise are presented in the popular science investigation by Oleksiy Kovalenko [11]. The analysis of the vocabulary and stylistic devices of Gargilius' text is the focus of articles by Maire Brigitte [12] and Dimitrios Mantzilas [13]. Recognizing the earlier works, we believe that the issues of generalizing the experience of practical use of vegetables in the prevention and treatment of various diseases and injuries, and maintaining health require additional coverage, since the principles of modern naturopathy and nutrition are based on the centuries-old experience of ancestors, and the study of the past of medicine gives an idea of the gradual development of skills in the treatment and prevention of diseases and understanding of the functions of the human body.

In antiquity, doctors have already noticed that the consumption of vegetables, which are an important source of vitamins, mineral salts, organic acids, pectin substances, dietary fiber, and phytoncides, had a beneficial effect on the human body, enhanced the secretion of the gastrointestinal glands, increased the digestibility of food, and strengthened the immune system. Having seen, for example, the laxative, emetic, diuretic, analgesic, and other effects of certain vegetables, doctors began to use vegetable plants not only to satisfy hunger but also to alleviate some or other health problem. Therefore, we can safely claim that the first medicines have their origin in the kitchen.

Let us consider the medicinal properties of vegetables on the basis of Gargilius' *Medicinae ex oleribus et pomis*, which consists of two parts. The subject of our study is the first part (chapters 1-39, 651 lines), which is devoted to the characterization of vegetables and spices. Gargilius' treatise is a compilation of ancient physicians' knowledge about the use of vegetables, spices, and fruits in medical practice, since the text of the work contains references to more than 20 authors, among whom Pliny the Elder, Dioscorides, Galen, and Columella are most often mentioned. As a result of the text analysis, it was found that Roman doctors used mainly those vegetables for medicinal purposes that

were available to ordinary Romans, namely black radish (Raphanus sativus), beetroot (Beta vulgaris), pumpkin (Cucurbita pero), cucumbers (Cucumis sativus), cabbage (Brassica oleracea), celery (Apium graveolens), parsnip (Pastinaca sativa), onion (Allium cepa), garlic (Allium sativum), horseradish (Armoracia rusticana) etc.

The analysis of the treatise showed that the medicinal properties of vegetables were most often used in surgical practice and traumatology. A variety of powders, ointments, and solutions have long been used to facilitate wound cleaning and healing, as well as to treat animal bites, due to their antimicrobial, anti-inflammatory, analgesic, dehydrating, necrolytic, and osmotic properties. Ancient doctors highly valued the antiseptic and bactericidal properties of onions, garlic, horseradish, leeks, and pumpkin. Thus, a powder made from crushed dry pumpkin seeds was used to fill wound defects: semini sicco ... in pulverem tunsum et inspersum vulnera quae cavata sunt (VI, 13)[1], and ashes of pumpkin peel contributed to the healing of purulent ulcers on the genitals: cinerem ... vulnera in veretro iam in putredinem versa purgare et ad cicatricem usque perducere (VI, 10) and the treatment of burns: cinis aridae cortices efficaciter potest combusta sanare (VI, 9). The experience of using pumpkins to treat surgical pathologies has been partly adopted by modern folk medicine [14]. Fresh pumpkin juice or grated pumpkin is used to make applications for burns and ulcers, and pumpkin lantern flowers are used to treat wounds. To cleanse fresh and old purulent wounds, it is recommended to wash them twice a day with warm water and make applications of raw, shredded cabbage: vulneribus ... recentibus ... veteribus, canceratis, ... crudam contritam bis die imponi (XXX, 8). In the modern practice of folk healers, the antiseptic properties of cabbage leaves are an addition to the basic treatment of burns, boils, and purulent wounds [14]. The application of a powder made from the root of burnt horseradish*:* radix adusta et in pulverem versa siccandis ac purgandis vulneribus imponitur (XXXII, 5) or the application of leek leaves with honey also helped to clean and dry wounds: folia contrita cum melle vulneribus apposite non minus profuerunt (XXI, 14). Eating leeks accelerated the process of bone fusion in fractures: fracturas alligat (XXI, 21). Roman doctors used garlic with lard as an antineoplastic agent: tumores suspectos ex adipe dissoluit (XVIII, 21). In case of burns, it was recommended to lubricate the affected areas until blisters appeared with lettuce leaves ground with salt: tritae cum sale combusta sanant, si ante pustellas inlinantur (XI, 6).

Topical application of green pumpkin peel with bread: ignis sacris cum pane subveniunt (VI, 12) or beets with alum allowed quickly relieve swelling, progressive inflammation of the skin and pain in case of erysipelas: ignes sacros et quae adusta sunt cum alumina inlita restinguit (X, 2).

In ancient times, bites from domestic and wild animals often caused wound infection and infectious diseases. In this regard, a number of recipes in the treatise are devoted to the treatment of bites. For example, the use of garlic or smearing wounds with garlic juice was recommended for snake and scorpion bites to cleanse wounds and accelerate their healing: serpentes et scorpiones ... fugat, ictibus eorum aeque medetur sive vulneri inlitum sive in potione vel in cibo sumptum (XVIII, 4), and with honey for dog bites: morsui canino cum melle succurit (XVIII, 5). Interestingly, cucumber leaves ground with wine were also used to heal wounds from dog bites: folia ... ex vino trita vulneribus medentur quae caninus morsus impresserit (XVI, 2).

The use of vegetable crops in the treatment of digestive diseases has a long history, largely due to their ease of use and direct effect on the organ. A number of recipes found in Gargilius' text are devoted to the treatment of problems of excessive gas formation in the gastrointestinal tract, diarrhea, constipation and colic. These pathologies were caused not only by non-compliance with sanitary and hygienic cooking standards, but also by digestive disorders and intestinal inflammation caused by bacteria, viruses and parasites. For example, diarrhea was treated with pumpkin seeds and wine: ex vino bibendum dederunt ut solutiones alui fluentis inhiberet (VI, 14-15) or hot drink made from roasted rutabaga seeds: semen eius tostum atque tritum bibendum ex aquae calidae cyatis quattuor dysentericis obtulerunt (XXXIV, 4); in case of colic, it was recommended to eat asparagus with butter, cumin and salt on an empty stomach: cum cymino, oleo, sale ... coli dolorem ... emendat, ... ventrem leniter mollit (XXXI, 3). The laxative properties of pectin substances in beets, leeks, and cabbage were widely used to treat constipation. Cabbage juice with iris and soda was effective for bowel cleansing: sucum eius viridem cum iri et nitro ad molliendum alvum (XXX, 23), beets with honey: iniecta cum melle onera ventris exponit (X, 4). Garlic with coriander and figs promoted the process of digestion: cum coriandro et fico datum, alvum mollire perhibetur (XVIII, 15) as well as leeks boiled twice in water: capita bis aqua mutata cocta molliunt alvum (XXI, 8). To prevent digestive disorders caused by changes in the place of residence, climate and water quality, it

[1] The Roman numeral stands for the number of the chapter, the Arabic numeral – for the number of the verse line.

was recommended to use garlic, which suppressed fermentation processes, prevented bloating and improved intestinal microflora: peregrinantibus esui datum minime patitur eos aquarum et locorum mutatione turbari (XVIII, 9). A mixture of seeds and shoots of celery, anise, and henbane was known as an antibacterial preparation against stomach bacteria: stomachum inundatio capitis laborantem mire tuetur (II, 9-10).

Long ago, people learned to counteract various poisons. As soon as humans began to eat food, their digestive system became vulnerable to foods of poor quality or contaminated with microorganisms. In Gargilius' work, we also find information on the treatment of toxic infections. For example, eating radish on an empty stomach was considered one of the best antidotes for poisoning: contra venena unicum remedium est (I, 7), and leeks were recommended for mushroom poisoning: in cibo sumptus plurimum prodest contra venena fungorum (XXI, 20). The most powerful antidotes for snake bites were rutabaga seeds: laudatissimi seminis et contra venena fortissimi (XXXIV, 6), a decoction of celery leaves or roots: efficaciter pugnat contra insidias venenorum (II, 5); turnip seeds in wine: semen ex vino vulneribus venenatis inlitum prodest (XXXV, 10) or leeks with pure wine or honey water: contra ictus venenatos vel cum mero vel cum aqua mulsa sucus datus prodest (XXI, 13). Horseradish juice mixed with wine showed an antitoxic effect on the body in case of scorpion bites: sucum eius ex vino datum contra venena utilem crediderunt (XXXII, 9).

Lack of hygiene, poorly processed food, in particular meat and fish, unwashed greens, vegetables and fruits, often caused worm infestations, which negatively affected human digestive tract due to dysfunction of the hepatobiliary system and intestinal mucosal barrier, caused metabolic disorders, reduced the concentration of vitamins and minerals and increased the susceptibility to infectious diseases. In case of tapeworm infection, Gargilius recommends garlic boiled with honey and vinegar: tineas et alia ventris animalia in mulso aceto coctum perimit et expellit (XVIII, 6). The natural bitterness of radish suggested its use with a pinch of salt as an anthelmintic: ventris animalia extinguit (I, 10).

Ancient medical sources also provide recipes for using vegetables as diuretics. Consumption of vegetables has a mild diuretic action on the body and does not cause side effects. Celery, cucumbers, asparagus, horseradish, rutabaga, and leeks helped to remove excess fluid from the body by stimulating the function of the kidneys. Celery, rich in sodium and potassium, was especially valued as a pharmaceutical diuretic that helped to discharge surplus liquid: nulla enim alia res fortius vel urinae difficultates resoluit (II, 2). Celery is still successfully used for this purpose [14]. A diuretic effect was also noticed in asparagus seeds mixed with cumin: semen obolorum trium pari pondere cum cymino potui datum … sollicitat urinam (XXXI, 10); horseradish: in cibo sumpta copiosam effundit urinam (XXXII, 1), which in modern folk medicine is successfully used to treat urolithiasis and cystitis due to its diuretic properties [14]. Radish helped to excrete surplus liquid from the body and normalize water-salt balance: urinam citat (I, 10). Since ancient times, it has been recommended to consume cucumber seeds rich in sulfur and silicon with sweet wine to treat urolithiasis: semen ex dulci vino datum vesicae laboranti facilem praestat urinam (XVI, 3) and a kind of rutabaga whose flowers are similar to those of anise: quod angulosis foliorum caulibus ad anethi similitudinem floret, vesicae laboranti ... utile existimant (XXXIV, 2). One of the most effective means of dissolving kidney stones was grated asparagus roots with white wine: radix ex vino albo trita et in potionem perducta calculos frangit (XXXI, 5).

The ancient Romans also used vegetables in gynecological practice. They successfully treated menstrual irregularities with vegetables. For example, in case of delayed menstruation, it was recommended to add a lot of onions or a mixture of onion juice and wine to food: tardantibus menstruis feminarum non inutile est vel ipsas in cibo offerre vel sucum ex vini potione miscere (XXXVII, 16), cabbage: purgationem feminis excitat (XXX, 16) or horseradish: incitat etiam menstrua feminarum (XXXII, 2). Rutabaga boiled in honey water was used to stop menstrual bleeding: cessantibus menstruis feminarum decoctum ex aqua mulsa utile existimant (XXXIV, 2). One can also find prescriptions for the treatment of uterine diseases and recommendations for increasing lactation. For example, grated asparagus root with white wine was recommended for uterine diseases: medetur etiam vuluae querelis (XXXI, 7), and raw cabbage with vinegar as an abortifacient for fetal death: cruda ex aceto sumpta … partus etiam mortuos pellit (XXX, 18). Regular consumption of cabbage: copiam lactis infundit (XXX, 16) or lettuce: lacte plurimo nutrients feminas implent (XI, 3) helped to increase lactation.

General medicine successfully dealt with fever, which in ancient times was understood as an acute febrile illness, by rubbing the abdomen with an ointment made from boiled mashed cabbage, butter and rose oil: cocta, contrite et stomacho cum axungia vetere et rosacio superposita, ardores febrium mitigat (XXX, 16) or by the patient rubbing with pumpkin juice and rose oil: rosacio mixtus et unctioni corporis admotus febrium restinguit ardores (VI, 8).

In case of cephalgia, one of the most common symptoms of various diseases, it was recommended to

lubricate the temples with soaked boiled garlic: elixum inlitumque temporibus dolori capitis opitulatur (XVIII, 17) or to instill leek juice with a third of honey in the ears or nose: profuit multis in dolore capitis, cum dormitum ire cupissent, idem sucus sive auribus sive naribus cum parte tenia mellis infusus (XXI, 17). Drops based on leek juice had a soporific effect on the body. Another remedy for insomnia was to eat cabbage boiled with salt and butter on an empty stomach: insomnia ac vigilias compescere si decocta cum sale oleo ieiunis in cibo detur (XXX, 10). Attacks of gout, which in ancient times was called the disease of kings, and joint inflammation were treated with compresses based on cabbage, vinegar and Greek flour: podagricis et arthriticis cum aceto et feni Graeci farina utiliter imponitur (XXX, 24) or turnips baked and grated with fat: tosta et cum adipe contusa articulorum dolori medentur (XXXV, 6).

The treasures of ancient Roman medicine can also provide us with many valuable recipes for treating respiratory and ENT diseases. Coughing is one of the important defense mechanisms of the human body that helps to free the airways from pathogens, dust, and various allergens. Vegetable crops contributing to gentle cleansing of the respiratory tract, mucus removal, and acceleration of recovery were successfully used as a cure for this symptom. Ancient medicine had an arsenal of proven remedies that were effective in treating coughs. For example, radish seeds and honey were considered to be an effective remedy for coughing: semine raphani cum melle trito … tussis arcetur (I, 9). Modern herbalists often recommend radish juice with honey as an expectorant for bronchitis [14]. The anti-inflammatory, antiviral and expectorant properties of garlic combined with beans have been successfully used to treat coughs and purulent inflammation in the chest: tussim et suppurationes pectoris mitigat in faba coctum (XVIII, 18). For thousands of years, the bactericidal, antimicrobial and antifungal properties of garlic have been highly valued by folk medicine. Garlic and honey milk, garlic with honey and lemon, and garlic water are successfully applied not only to soothe coughs and improve sputum production, but also to treat tuberculosis, physical fatigue, and to strengthen the immune system. Another indispensable and effective remedy for treating a long-standing cough was a mixture of leek juice and breast milk: eodem succo plerique cum lacte mulieribus veterem tussim atque pulmonis vitia sanarunt (XXI, 15) or barley soup with leeks: in sorbitione ptisinae datus … tussim, thoracis, arteriae pulmonisque vitia compescit (XXI, 6). To thin sputum, Roman doctors often recommended horseradish with honey: pituitas glutinosas cum melle sumpta dissoluit (XXXII, 4), which is still considered a natural antibiotic with pronounced antibacterial properties. In the treasury of folk recipes, a mixture of horseradish and lemon juice is stored as a mucolytic agent [14].

Roman therapists successfully treated diseases of the nasopharynx and ears with vegetables. For example, due to the high content of biologically active substances and micro- and macronutrients, radish juice with rose oil was recommended for prevention of hearing loss: succus gravitatem aurium … emendat (I, 5), and the anti-inflammatory properties of garlic juice with goose fat helped relieve ear pain better than any other medicine: aurium doloribus sucus cum adipe anserino tepefactus infunditur (XVIII, 18). Leek juice with one third of honey was also considered an effective remedy for otitis media and nasopharyngeal diseases: idem sucus sive auribus sive naribus cum parte tenia mellis infusus (XXI, 17), drops made from onion juice and breast milk: instillatus auribus sucus cum lacte mulieris prohibit dolorem (XXVII, 13) or pumpkin juice: ad compescendum velociter auriculae dolorem tepefactus infunditur (VI, 6). In case of dysphonia, ancient medicine recommended garlic cooked with peas or beans: raucitatem vocis emendat in pisa aut in faba sumptum (XVIII, 19), leeks with barley porridge: in sorbitione ptisanae datus … vocem purgat (XXI, 7).

It is noteworthy that among numerous recipes for vegetable treatment, we find advice on the prevention of ophthalmic diseases. To relieve conjunctival edema, ancient medicine recommended applying grated pumpkin to the eyes: rasura earum oculorum tumores tollit (VI, 31), and regular consumption of cabbage helped prevent pathological disorders caused by sudden blurred vision and loss of image clarity: cibus eius assiduus caliginem discutit (XXX, 16).

Vegetable-based medicines were also widely applied in dermatology to treat alopecia, seborrhea, scalp ulcers etc. The treatise pays special attention to the problem of treating hair loss and baldness, as healthy and good hair has always been considered a way of manifesting beauty and attractiveness. Thus, medicines based on garlic, onions, cabbage, and beets were successfully used to treat alopecia and seborrheic dermatitis. For example, in case of ulcers on the scalp and alopecia, pounded raw beets were used: ulceribus in capite manantibus item alopeciis cruda trita optime inlinitur (X, 3). In ancient medicine, radish mashed with honey was used externally to fight alopecia: Capillos alopeciae iniuria raptos cum melle trita restituit (I, 4), and the juice was used against pediculosis: contra pthiriasim sucus eius adhibetur (I, 7). In modern folk medicine, radish oil is used as an insecticide for pediculosis [14]. To restore and accelerate hair growth, Gargilius recommends applying onion gruel on the scalp: alopecias tunsis cepis infricare inter efficacia remedia compertum est

(XXVII, 19), and against scabies, rubbing the skin with cabbage soaked in vinegar and alum: lepras et psoras cum alumine rotundo ex aceto inlita emendat. Eadem virtute … fluentes capillos retinet (XXX, 20-21). Masks made from onion juice, which stimulates blood flow to the hair follicles and enriches the skin with useful microelements, are still in the arsenal of folk recipes for combating hair loss and baldness [13]. To fight seborrhea, wash hair with water in which beets were boiled: aqua in qua decocta est furfures capitis elimat (X, 4). Pain and discomfort from foot abrasions were treated by applying a vegetable compress made of grated raw turnips: pedum maximeque trituris cruda tunsa succurrunt (XXXV, 9).

Since ancient times, people have had dental problems. The reason for this was the lack of proper oral hygiene, the use of solid food, and vitamin deficiency. Dentistry in Rome was not only about removing affected teeth. The treatise contains information about how Roman doctors tried to preserve patients' teeth conservatively. For example, to relieve toothache, they recommended keeping warm pumpkin juice in the mouth: idem sucus et dolores dentium mitigat, si diutius in ore teneatur (VI, 7). Toothache relief was also believed to be achieved by rubbing the teeth with parsnip root: dentes redice pastinacae circumscalptos credunt a dolore relevari (XXXIII, 8). Rinsing the mouth with onion juice also helped to strengthen teeth: conluendis dentibus frequenter assumptum integros eos servare praesumitur (XXVII, 15), and eating onions with bread remedied stomatitis: medentur oris ulceribus, si cum pane manducantur (XXVII, 8). It should be noted that even today, doctors recommend to consume a lot of onion, which has a powerful antimicrobial effect and destroys bacteria that cause caries and gum disease [14].

Certain vegetable crops that can increase sexual desire have been successfully used in the treatment of sexual disorders. Roman doctors already noticed that parsnips and asparagus are natural aphrodisiacs and have a positive effect on marital relations. Daily consumption of parsnips, rich in essential oils, increased sex drive and libido: venerem stimulat copiosior in cibo sumpta (XXXIII, 7). Aphrodisiac powers of parsnip are recognized by modern doctors, too. Asparagus, which in Gargilius' time was recommended to be consumed with cumin, had a beneficial effect on male health, improved physiological tone, and helped maintain a good mood: semen obolorum trium pari pondere cum cymino potui datum venerem stimulat (XXXI, 10).

CONCLUSIONS

The ancient physicians left us a wealth of works that are now an invaluable source of information on the medical science of the time. One of these works is Quintus Gargilius Martialis' manual *Medicinae ex oleribus et pomis*, dedicated to the use of vegetables, herbs, fruits and nuts in the medical practice of Roman physicians.

Already in ancient times, the consumption of vegetables was considered one of the easiest ways to treat and prevent numerous diseases, and each vegetable crop was seen as a pharmacy in miniature. Due to the high content of biologically active substances, micro- and macroelements, fibre, phytoncides, essential oils, the Roman doctors highly valued the therapeutic and preventive properties of radishes, pumpkins, cucumbers, celery, beets, cabbage, turnips, rutabagas, lettuce, onions, garlic and other vegetables. Romans successfully used vegetable crops as medicine to strengthen immunity, enrich the body with vitamins and trace elements, as well as to prevent and treat wounds and injuries in surgery, various tumours and inflammations, gastrointestinal diseases, gynaecological pathologies, fever, cough, diseases of ENT organs and skin. In this regard, Hippocrates' statement that our food should be medicine and our medicine should be food remains relevant.

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REVIEW ARTICLE

A HEALTHY SOCIETY: SOCIAL CHALLENGES OF DIGITALIZATION AND THE WAYS TO OVERCOME THEM (THE UKRAINIAN EXPERIENCE)

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ABSTRACT

The aim: The article examines the peculiarities of the Ukrainian state policy in the field of digitalization, reveals the social challenges caused by this phenomenon, and outlines the ways to overcome them.

Materials and Methods: The data collection was carried out using PubMed, Scopus, Google Scholar databases. Research papers were identified according to the search terms: "digitalization", "digital transformations", "Internet", "digital services", "smart city", "smart urbanization", "inclusion", "social exclusion", "community mental health", "volunteering", "social partnership". The authors analyzed international and domestic official strategies, programs, and messages along with statistical data and social surveys conducted by foreign and Ukrainian institutions, public organizations, and analytical centers. The authors used the interdisciplinary approach along with the principles of objectivity, tolerance, and impartiality, and general scientific methods, such as induction, deduction, generalization, etc.

Conclusion: The rapid spread of digital technologies is associated with the growth of social cohesion, inclusion, solidarity, and the development of a healthy harmonious society that will provide all the conditions for a decent life for a human being and the comprehensive development of his/her abilities and talents. These hopes are not groundless, because digitalization is accompanied by a number of structural shifts in economics and public administration, which contribute to overcoming subjectivity in making management decisions and increasing the level of "intellectualization" of the environment. In addition, digitalization is becoming a significant driver of the sustainable growth in labor productivity, employment levels, personal and social well-being; and the spread of digital technologies provides an opportunity to overcome various social challenges. As the Ukrainian experience reveals, despite a number of positive shifts, digitalization can also give rise to destructive social trends, among which the digital gaps caused by the uneven access to digital technologies and services occupy a special place. People in the city outskirts, small towns, and especially in the remote rural areas often have extremely limited access to the Internet that significantly reduces their social opportunities. These problems became more acute after the full-scale invasion of the russian federation into Ukraine. The occupation of the part of Ukraine, hostilities, and missile attacks damaged the energy sector blocking telecommunication networks, which led to the social exclusion of a significant part of the population in some Ukrainian regions. The harsh living conditions during the war, the social exclusion as a result of the occupation, as well as the destruction of energy infrastructure and civilian objects fueled the activities of the Ministry of Digital Transformation of Ukraine. The proposed services and transformations provided social opportunities for a part of the population, while remaining unable to overcome social exclusion generated by the digital, social, or other gap. Volunteers and social activists usually help to bridge the gap and maintain mental health of the community, which has been suffering from the horrors of the war for more than a year. Their activities and public position lay the ground for the establishment of social partnership aimed at the harmonious development of every individual and the community as a whole.

KEY WORDS: digitalization, digital transformations, Internet, digital services, inclusion, social exclusion, community mental health, volunteering, social partnership

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INTRODUCTION

Global socio-cultural transformations that began in the middle of the 20th century cast doubt on all previous ideas and worldviews. According to Toffler, this situation is quite natural due to the fact that the "old ways of thinking, old formulas, dogmas, and ideologies, no

matter how cherished or how useful hi the past, no longer fit the facts. The world that is fast emerging from the clash of new values and technologies, new geopolitical relationships, new lifestyles and modes of communication, demands wholly new ideas and analogies, classifications and concepts" [1, p. 7]. The scientist coined the rapid creation of a new world order as the "third wave" of those civilizational transformations, which, in his opinion, will manifest themselves throughout the world transforming the established social system.

Toffler's book "The Third Wave" was published when the changes he proclaimed were only gaining momentum, and therefore the scientist could only outline the image of the future world. The changes, both predicted and unforeseen by Toffler, have already become commonplace in many, especially developed, countries, and have transformed all spheres of public life. Informatization and digitalization have become their main drivers. Although they have not yet gained full momentum, they are rapidly modifying economies and societies forming a digital environment that transforms the everyday life of the individuals: "A new civilization is emerging in our lives, and blind men everywhere are trying to suppress it. This new civilization brings with it new family styles; changed ways of working, loving, and living; a new economy; new political conflicts; and beyond all this an altered consciousness as well. Pieces of this new civilization exist today. Millions are already attuning their lives to the rhythms of tomorrow. Others, terrified of the future, are engaged in a desperate, futile flight into the past and are trying to restore the dying world that gave them birth" [1, p. 11]. The latter try to restore the "old world" despite the fact that digitalization and informatization have opened many doors of opportunity for business activity and social life in general. For example, the use of modern technological capabilities is becoming a key factor in production; it enhances the competitive advantages of enterprises opening up access to foreign markets. Digitization is becoming an important driver of sustainable growth of workforce productivity, employment level, and general welfare. The spread of digital technologies contributes to reducing the level of environmental pollution, overcoming challenges in the field of health care, education and public administration, etc. [2, 3, 4].

Nowadays, the use of digital technologies is seen as the way to create a more inclusive, environmentally responsible, and cost-effective environment. This is evidenced by the priorities and strategic goals defined by the European Union. Let us recall the industrial policy strategy "Investing in a smart, innovative and sustainable industry" published in 2017 [5], the purpose of which was to search for mechanisms to ensure the competitiveness of industry, to increase its digitalization level and to use the best technologies for a smart, clean, and innovative industry of the future for the revitalization of the regions and, consequently, the empowerment of the EU citizens. In the same year, the "Strengthening Innovation in Europe's Regions: Strategies for resilient, inclusive and sustainable growth" was elaborated by the European Commission. The "Strategies" declared the program of smart specialization for European regions and proposed pilot measures to activate their potential in the social innovations' development [6]. In "A renewed European agenda for research and innovation – Europe's chance to shape its future" (2018) [7], innovation and digitalization were recognized as the main prerequisites for maintaining the European way of life and well-being of millions of people in Europe, primarily due to the competitiveness of key industries. For this purpose, in 2021, the European Parliament and the Council of the European Union approved the "Regulation Establishing the Digital Europe Program" [8], which established a financial package for the implementation of the "Digital Europe" Program for the years 2021-2027. Thus, the European Union has clearly and unequivocally recognized that digital transformation is a fundamental factor in preserving well-being and cultural diversity in Europe.

The positive global experience of using digital technologies in all spheres of social life has not gone unnoticed by Ukrainian society and even the Ukrainian authorities. Trying to develop services that would meet the needs of the Ukrainians and contribute to the growth of social well-being of Ukraine, in September 2019, the Cabinet of Ministers of Ukraine established the Ministry of Digital Transformation of Ukraine. It is aimed to form and implement state policy in the field of digitization, open data, and national electronic information resources. In this regard, the ministry's roadmap covered the period up to 2024 and intended to transfer 100% of all public services for citizens and businesses online; to provide 95% of the public transportation, cities and villages and their social facilities with access to high-speed Internet; to teach 6 million Ukrainians digital skills; to increase the share of IT in the country's GDP up to 10% [9]. Within a year, in April 2020, a mobile app, a web-portal and a brand of e-governance in Ukraine "Diia", which offered 27 public services online, was officially launched [10].

Urbanization has become no less relevant trend. Today, cities are becoming the dominant form of social and economic organization, often ahead of entire countries in terms of their development. This is due to the changes that had taken place in the economy during the last century. Until the end of the 20th century, the

economic growth of the cities was closely related to industrialization, while in recent years those who has invested in intellectual capital and the development of creative sectors have won the competition [3; 11]. In other words, the economic focus on innovation and digitalization contributes to the development of the cities, especially due to the concentration of intellectual services in the big cities. This is evidenced by the policy of high-tech companies, which, despite the developed networks and communication channels, prefer big cities that can provide live communication with partners and customers. Obviously, that is why Kyiv, Kharkiv, Lviv, Dnipro, Odesa, Vinnytsia and Zaporizhzhia have become the largest Ukrainian IT centers [3; 12].

Therefore, the rapid growth of the cities in the modern world creates challenges that can be overcome in the process of transition to the smart cities, which, according to the Irish professor Kitchin, appears as an attempt to solve the major global issues, namely, to reduce costs simultaneously increasing economic growth, ensuring stability and acceptability of public services, and maintaining a high level of quality of life of the population [13]. To put it another way, smart urbanization is focused on the introduction of innovations, the development of inclusiveness, justice, and the involvement of every city dweller in the "smart society". The specified changes will contribute to the qualitative renewal of the community, which will gradually be transformed from a socially amorphous group situationally related by a common place of residence into an integrated community, in which relations are based on transparency and mutual trust. At the same time, the "smart arrangement" of urban space should contribute to the growth of the community's quality of life; optimize spending on infrastructure; strengthen the safety of community members; positively influence the "investment image" of the cities thereby strengthening their tourist potential [3, p. 285]. Thus, the development of smart cities has great economic and social prospects, which can contribute to the creation of a harmonious and healthy urban environment with a high level of well-being of the population.

Despite the fact that digitalization and "smart" urbanization promise many positive social changes, the rapid development of technologies is accompanied by various doubts and numerous caveats [3, 14, 15], which seems to indicate the contradiction of the transformations caused by the Fourth Industrial Revolution. One of the most acute problems caused by digital transformations is social and financial "gaps", which have become quite obvious in recent years [16]. "Smart" urbanization, which has revealed the problem of the digital and economic "gaps", as well as socio-economic inequality between cities, within the cities themselves, and between the urban and rural population, is no less worrisome [3, p. 10]. The researchers are concerned with the possible "displacement" of the representatives of certain professions, which in the future may significantly deepen the digital "gap" and lead to the growth of social inequality [3, p. 10] and violation of the right to privacy due to total surveillance [15].

Thus, the spread of digital technologies is expected to increase social cohesion, inclusiveness, solidarity, and the development of a healthy harmonious society. At the same time, the extremely rapid development of digital reality increases the risk of growing social inequality and, consequently, exclusion as an important indicator of individual and public health. It is clear that the risks cannot stop changes; therefore, any society should create and develop mechanisms to overcome the social challenges of digitalization in order to maintain mental health of the society as a guarantee of peace in the world.

THE AIM

The article examines the peculiarities of the Ukrainian state policy in the field of digitalization, reveals the social challenges caused by this phenomenon, and outlines the ways to overcome them.

MATERIALS AND METHODS

The data collection was carried out using PubMed, Scopus, Google Scholar databases. Research papers were identified according to the search terms: "digitalization", "digital transformations", "Internet", "digital services", "smart city", "smart urbanization", "inclusion", "social exclusion", "community mental health", "volunteering", "social partnership". The authors analyzed international and domestic official strategies, programs, and messages along with statistical data and social surveys conducted by foreign and Ukrainian institutions, public organizations, and analytical centers. The authors used the interdisciplinary approach along with the principles of objectivity, tolerance, and impartiality, and general scientific methods, such as induction, deduction, generalization, etc.

REVIEW AND DISCUSSION

In recent years, certain shifts toward digitalization have been observed in Ukraine. The most important milestone was the introduction of the program the "State in a Smartphone" in 2019 [17]. Thus, Ukraine became the first country in the world to legalize the electronic

passports (e-Passports) and equate them with the paper ones. In addition, the introduction of this program made it possible for Ukrainians to change their places of registration online; opened access to the tax services; provided the opportunity to register businesses remotely, submit direct appeals to the state in the form of electronic petitions, sign documents remotely using e-signatures; etc. According to the Ministry of Digital Transformation of Ukraine, the Ukrainian authorities do not plan to stop there promising to bring all state services online by 2024 [18].

Already implemented and announced projects of the digital transformation of Ukraine may be seen as an important and positive step on the way towards the state without corruption, bureaucracy, queues, and, importantly, "without papers". As the Minister of Digital Transformation of Ukraine Mykhailo Fedorov stated on May 18, 2021 at the All-Ukrainian Forum "Ukraine 30. Digitization", they provide "an opportunity to ensure equal access to resources for all Ukrainians", making the life of every Ukrainian more convenient [19]. The Law № 1689-IX regarding electronic public services, which was adopted by the Verkhovna Rada of Ukraine and signed by the President of Ukraine in August 2021, should contribute to it [20]. It defines the legal basis for exercising the rights and freedoms of individuals and companies in the field of providing electronic public services and cancels "unnecessary documents" that are stored in the registers of the authorities. Actually, the paperless mode implies receiving special services and information online.

Simultaneously with the transformation of Ukraine into the digital state that meets the needs of people, the process of developing smart cities is underway. Around the world, the "smart" urbanization began in the early 2000s, and in Ukraine – only in the last five to seven years. At this time, the Ukrainian local authorities started showing interest in projects that increase the level of "intellectualization" of the urban environment and introduced facial recognition technologies, contactless payment in retail and public transportation. Intelligent traffic lights became a welcomed novelty. Many cities have smart water and gas meters, and the use of the smart garbage containers is only gaining popularity [3, p. 136].

Nowadays, an increase in the level of "intellectualization" of the urban environment may be seen as a clear trend. The "smart projects" are being developed by the teams of specialists in Kharkiv, Lviv, Dnipro, Vinnytsia, Poltava, Drohobych, Kyiv, Mukachevo, etc. E-democracy and city management, education, medicine, ecology, urban mobility, public safety, etc. are becoming the main areas and directions of the digital transformation [3, p. 136-145; 21]. The speed and efficiency of the implementation of the smart infrastructure development projects in different cities varies significantly and depends on the city's economic potential, budgetary support, and the scale of public support.

Despite Ukraine's considerable achievements in the digitalization of all spheres of social life, in 2022, according to the E-Government Development Index, Ukraine ranked 46th out of 193 UN member countries [22]. It should be emphasized that for Ukraine, which ranked 69th in 2020, this is a noticeable shift, however, it can hardly be called sufficient given that Ukraine is the second largest IT services export country in Europe [3, p. 152]. Such a slowdown in the development of digital trends in Ukraine, which are extremely necessary, especially during the russian federation's ongoing aggression against Ukraine, is due to a number of obstacles. And one of them is the rather low level of incorporation of the state institutions in the implementation of the Concept for the Development of the Digital Economy and Society. The inconsistency of relevant legislation with global challenges and opportunities, the weak state policy regarding the development of the innovative economy, and a rather low level of motivation of the government institutions, which, according to the experts of the Ukrainian Institute of the Future (at least in 2018), did not have a complete understanding of the benefits of total digitalization, are considerable obstacles on this path to the better future [23]. Obviously, the high level of populism regarding digitization projects does not contribute to the introduction of the "smart" innovations. For instance, we can mention the "paperless mode" introduced by the Ministry of Digital Transformation of Ukraine, the implementation of which at the level of the local United Territorial Communities (UTCs) without the systemic state support was extremely problematic even in the pre-war period [24], but in the conditions of the crisis caused by the war, it is transformed into the project that is unlikely to be implemented.

The unpreparedness of the United Territorial Communities for the introduction of digital technologies is quite natural, at least, considering the state of Internet coverage in Ukraine. For example, in 2020, the situation with fiber optic Internet in the cities of Ukraine was quite good due to the fact that several providers were based here, which ensured competition in the market. On the other hand, the access to the Internet in rural areas was worse: more than 4 million Ukrainians lived in the villages without high-quality fixed Internet, and 1.55 million lived in remote areas, where there were fiber optic Internet providers, but the cost of the services exceeded 150% of the average market value [25].

Significantly inflated tariffs of Internet providers for rural residents, who, according to the State Statistics Service of Ukraine, had a slightly lower level of income than the urban population [26, p. 14], significantly limited access to the network thereby creating digital "gaps" in Ukrainian society.

The elaboration of the National Strategy for the Development of Broadband Access to the Internet in Ukraine was the answer to these problems. Understanding a low return on investment in networks due to the small number of subscribers, the state proposed to use alternative infrastructure for telecommunication networks (motorways, railway tracks, power lines, sewage networks, heat supply networks, water supply) and to provide fixed broadband Internet access in remote settlements connecting social facilities (schools, hospitals, libraries, etc.) to fiber optic networks. In this way, they hoped to provide 95% of the population of Ukraine with 100 Mbps broadband Internet speed for by 2024 [27].

The development of the "road map" with the clearly defined program of actions and deadlines for their implementation did not significantly improve Ukrainians' access to high-speed fixed and mobile Internet. Thus, according to the data of the Department of Education and Science of the Kyiv City Council, in 2020-2021, 72.4% of computers in Ukrainian schools were connected to the Internet. At first glance, this is not a low indicator, but if we take into account the fact that the average Internet speed in secondary education institutions is in-between 30 and 100 Mbps and only 25% of these educational institutions reach the level 100 Mbps or more, the situation looks different. Such an indicator is quite interesting, given the fact that according to the state standards [29] in order to ensure high quality distance learning in quarantine conditions, secondary education institutions must be provided with 100 Mbps Internet speed with the possibility of shifting to a tariff plan "1 Gbps Internet speed" without network equipment upgrade. Moreover, families with students and teachers must have the technical ability to connect to 100 Mbps Internet at market prices. This implies that the communities where these families live are provided with fiber optic networks [29].

In 2020, according to the Ministry of Digital Transformation of Ukraine, 40% of Ukrainian schools did not have access to Internet [29]. Given this fact, it is difficult to talk about high-quality school education in remote regions of Ukraine in the conditions of long-term quarantine [30]. Due to the lack of proper access to high-speed Internet, these children found themselves in informational and educational isolation, which gave rise to social exclusion of a significant part of Ukrainian youth. Unequal access to social opportunities (in particular, to the system of social services) and the lack of necessary social conditions for human functioning [31, p. 145] have extremely negative social consequences. The growth of social barriers and the deepening of the "social bottom" entails social confrontation, which is manifested not only verbally, but also in destructive social activities, such as alcohol and drugs abuse, the rise of crime, etc. Besides, people who have limited access to social opportunities demonstrate a low level of civic self-organization, and therefore they can be puppets in the hands of politicians who use the electoral system to realize their own political ambitions [32, p. 6-8].

Unequal access to digital resources in Ukraine causes social exclusion not only of a part of rural youth, but also of a large number of elderly people in both villages and cities. This situation arises as the result of the extreme poverty of Ukrainian pensioners. Many of them have an average monthly income of less than $100 [33]. Living below the poverty line does not provide the opportunity to purchase the gadgets necessary for a digital society, thus depriving these people of proper access to public resources. For example, in the conditions of digitalization and the development of smart cities, the lack of gadgets entails considerable difficulties for the elderly in using the most necessary resources for survival, such as public transportation, medical assistance, electronic government, social service, etc. Thus, many Ukrainian pensioners are subjected to social exclusion, being deprived of access to the social benefits necessary for survival. This often leads to the situations in which these people become victims of various pre-election manipulations by immoral political elites, the purpose of which is to preserve their own political positions at the expense of socially excluded individuals in particular [32, p. 6-8].

With the beginning of the full-scale russian invasion in Ukraine in February 2022, the need for digitalization of public services began to grow rapidly. Due to the brutal actions of the russian military, many Ukrainians (usually in the de-occupied territories) need to restore documents certifying identity, ownership, special status, marital status, education, etc. Other people, and first of all, temporarily displaced persons, volunteers, military personnel, medical staff, people affected by rocket attacks, etc., are in dire need of digitalization. At the same time, military actions lead to an increase in the need for high-speed Internet, which is becoming one of the key requirements in ensuring the vital activities of the state and society in war conditions, because it facilitates and accelerates document circulation in the armed forces, registration of damaged property, communication with relatives, activities of volunteers and volunteer organizations, educational institutions, etc.

The growing need for high-speed Internet access has become a challenge for some Internet providers in Ukraine. Responding to the challenges of the times, they put a lot of effort into providing Ukrainians with high-speed fiber optic Internet in the winter of 2022-2023. In the conditions of long-term power outages, it was an important step in maintaining certain spheres of life of the urban population. People who had the financial and technical abilities to connect to these services maintained access to the most important social resources. Instead, the population of the regions that were defined by providers to be unprofitable for laying fiber optic cable (the city outskirts, small towns, villages, etc.) had extremely limited access to vital social resources, including emergency medical care. This period turned out to be especially difficult for people with low social opportunities, even in the pre-war period (low-income earners and elderly people, the population of remote rural areas, etc.). Given the absence of community involvement and the harsh conditions of martial law, they became socially excluded.

The horrible living conditions during the war, the social exclusion of a large part of Ukrainians as a result of the occupation, as well as the destruction of energy infrastructure and civilian objects fueled the activities of the Ministry of Digital Transformation of Ukraine. During the war, it has been actively engaged in digital transformations in different regions of Ukraine. Due to online services, some services have been provided throughout Ukraine. For example, the eMalyatko service – all-inclusive online service for registering newborn babies – has become quite useful for Ukrainians; and online services for internally displaced persons, volunteers and victims of hostilities, etc. have become no less effective [34]. However, the introduction of various online services (eOselya, eVorogh, eMalyatko, etc.), digital registries, catalogs, and other online services did not lead to a rapid increase in the level of inclusiveness of Ukrainian society, at least given the fact that part of the Ukrainian regions are still occupied by russian troops, therefore, they have extremely limited access to various state resources, including digital ones. The residents of the Kherson region, part of which is occupied by russian troops and part of which was flooded due to the destruction of the Kakhovka Dam, are also deprived of access to various social opportunities [35]. Certainly, after lowering the water level and restoring electricity and communication [36], these people gained formal access to basic state resources and services, but whether they can use them at the moment is difficult to say, because this requires personal digital devices. In their absence, people are gotten into a vicious circle – in order to receive financial support for the restoration of damaged property, it is necessary to certify the identity and record the degree of damage, which is impossible to do in terms of "paperless mode" due to property damage; and without recording the damage, it is impossible to receive financial support.

Unfortunately, there is no reason to hope for the immediate restoration of social inclusion of the Kherson population. And the point is not only that the terrible flood led to the death of many people, but it is also about the consequences of the destruction of the neighborhood and the very idea of the unity of the community. As Erikson [37] showed, this situation leads to the loss of the moral spirit, the anchors that determine the life of the individuals and the community as a whole. Activities that support everyone in everyday life – lifestyle, work, care, entertainment – lose their meaning outside the neighborhood community leading to the destruction of the established beliefs. From the standpoint of the existential experience of many victims, this situation may be seen as hopeless and total. The feeling that there is the end of the world and there is no good reason to do anything can lead to apathy and fatigue, as well as to such antisocial phenomena as alcohol and drugs abuse, and crime. As Erikson stressed, such antisocial behavior is a kind of attempt to bring the feelings back into the life distorted by the flood [37, 38].

Definitely, it is still difficult today to determine the scale of the disaster caused by the destruction of the Kakhovka Dam and the social consequences of the flooding of the part of the Kherson region. Meanwhile, in the process of eliminating the consequences of this tragedy, it is becoming obvious that digitalization is only one of the aspects of creating an inclusive society. The digital government services cannot respond to all social requests in peacetime, not to mention wartime. As modern Ukrainian realities show, socially active people concerned about the welfare of society, its problems and the solutions to these problems, as well as charitable organizations and volunteers try to overcome the bureaucratic formalism [39, 40, 41, 42]. It should be noted that the growth of the volunteer movement in Ukraine, despite the tradition of mutual help and care for the poor and suffering rooted in customary practices, took place in 2014 only and was caused by "the domestic political crisis that led to an imbalance in the public administration system, a lack of quality management decisions, a lack of resource opportunities, and external aggression that deepened the imbalance between the ability of the state to effectively perform its functions and ensure the basic needs of the citizens" [43, c. 48]. The inability of the state authorities to respond in a timely manner to the challenges caused a surge in public activity at the

national level. In a matter of a few months, volunteer initiatives of the groups of citizens were turned into powerful organizations, "whose activities were aimed at providing almost the entire spectrum of needs of both law enforcement agencies and citizens affected by hostilities" [44, p. 95]. Over time, the leaders emerged; they united these disparate self-organized groups and began to work under centralized leadership. Later they increased their influence on the government system and the effectiveness of aid and support [45, p. 9].

To date, 4,365 civil society organizations, which help military personnel, medical staff, displaced persons, and other Ukrainians to overcome the difficulties of the war, are officially registered in Ukraine [46]. The high level of the Ukrainian initiatives is also manifested in the form of spontaneous volunteering [47], which provides support to people in need. Therefore, volunteers mitigate the reduction in the role of the state in helping those in need, and, if possible, neutralize the social exclusion caused by the digital transformations and the war, etc. The establishment of a social partnership between the community, the volunteers, and the state is not possible without the democratization of all aspects of social life and the observance of human rights; therefore, it is an important prerequisite for the development of a healthy society.

CONCLUSIONS

The rapid spread of digital technologies is associated with the growth of social cohesion, inclusion, solidarity, and the development of a healthy harmonious society that will provide all the conditions for a decent life for a human being and the comprehensive development of his/her abilities and talents. These hopes are not groundless, because digitalization is accompanied by a number of structural shifts in economics and public administration, which contribute to overcoming subjectivity in making management decisions and increasing the level of "intellectualization" of the environment. In addition, digitalization is becoming a significant driver of the sustainable growth in labor productivity, employment levels, personal and social well-being; and the spread of digital technologies provides an opportunity to overcome various social challenges.

As the Ukrainian experience reveals, despite a number of positive shifts, digitalization can also give rise to destructive social trends, among which the digital gaps caused by the uneven access to digital technologies and services occupy a special place. People in the city outskirts, small towns, and especially in the remote rural areas often have extremely limited access to the Internet that significantly reduces their social opportunities. These problems became more acute after the full-scale invasion of the russian federation into Ukraine. The occupation of the part of Ukraine, hostilities, and missile attacks damaged the energy sector blocking telecommunication networks, which led to the social exclusion of a significant part of the population in some Ukrainian regions.

The harsh living conditions during the war, the social exclusion as a result of the occupation, as well as the destruction of energy infrastructure and civilian objects fueled the activities of the Ministry of Digital Transformation of Ukraine. The proposed services and transformations provided social opportunities for a part of the population, while remaining unable to overcome social exclusion generated by the digital, social, or other gap. Volunteers and social activists usually help to bridge the gap and maintain mental health of the community, which has been suffering from the horrors of the war for more than a year. Their activities and public position lay the ground for the establishment of social partnership aimed at the harmonious development of every individual and the community as a whole.

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REVIEW ARTICLE

FEATURES OF THE PROCESS OF TRAINING IN EDUCATIONAL MEDICAL INSTITUTIONS OF UKRAINE AT THE PRESENT STAGE. PART I. ATTITUDES OF STUDENTS AND UNIVERSITY TEACHERS TOWARDS DISTANCE LEARNING

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ABSTRACT
The review article reflects the results of the assessment of data from individual research papers and personal observations, as well as data from domestic and foreign literature, as well as own opinion about the features of the training process in educational medical institutions of Ukraine at the present stage. First, the COVID-19 pandemic, and then martial law in the state, caused distance learning to become an inseparable component of modern education and contributing to the effective implementation of the educational process in general and the achievement of high quality results in medical educational institutions in particular. For the successful implementation of the e-learning system, all participants in the educational process need the help and assistance of special technical support services. Therefore, universities need to pay more attention to improving technical support of platforms and academic interaction between teachers and students [1]. Universities that have been able to provide such support have avoided many problems in the transition to distance learning. Practical and clinical skills are essential in the field of medicine, and the lack of opportunities to master and practice these skills could potentially lead to a generation of insecure doctors with limited experience of meeting and examining real patients [2]. Therefore, the task of teachers at the present stage is to do everything possible to prepare competent specialists adapted to the realities of life.

KEY WORDS: educational medical institutions, medical students, COVID-19 pandemic, martial law, distance learning

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INTRODUCTION

The COVID-19 pandemic, and the public health measures that followed, the war conditions in the country have challenged medical universities and their traditional teaching concepts.

The COVID-19 pandemic has affected all areas of life, including education. According to some reports, in early April 2020, 170 countries closed their higher education institutions. About 1.7 billion students and students were unable to attend universities and schools, comprising 90% of the total number of all students on the planet [3]. The COVID-19 pandemic and the state of war have led to isolation, social distancing, and work from home, making online classes part of the lives of teachers and students.

At the present stage, online education has become an academic norm. Today, many universities maintain, to one degree or another, a distance learning format. This contributed to the organization of the educational process in the conditions of war. A certain part of students and teachers are in dangerous zones – in the territories of warfare or temporarily occupied territories and do not have the opportunity to join it. Conversely, there are students and teachers who are relatively safe, but do not have access to the Internet or do not have technical means. Every day (several times a day in some cities) in most regions of Ukraine, an air alert is announced, requiring residents to go to a shelter. And this directly affects the organization of the educational process [4]. In addition, such factors as the loss of material and technical base by some educational institutions have a negative impact; individual psychological state of a person during the war (combat operations, missing persons, wounded, dead, etc.); evacuation of some students and teachers, mobilization of direct organizers of the educational process, active volunteering of teachers and students. In order not to worsen the quality of education in medical universities, it is desirable to minimize the influence of these factors on the educational process [4].

It is generally recognized that the situation has become a serious test for the entire education system as a whole. There is uncertainty about how long this situation will last, and there is growing recognition that there may be periods in the future when quarantine and social distancing may be required again [5]. Based on the opinions of experts, it can be said that recovery from the pandemic may take several years, and given the military situation in Ukraine, this period will be even longer.

THE AIM

To characterize the learning process in educational medical institutions of Ukraine at the present stage

MATERIALS AND METHODS

This article presents an assessment of more than 70 world literary sources published in the period from 2020 to 2023, which discuss the issues of distance learning process at medical universities. The study involved an evaluation of literary sources included in the PubMed, Google Scholar databases.

The assessment employed data from sociological studies on the attitude of students and teachers of universities to distance learning, conducted by expert organizations during the pandemic, analytical and information materials of universities, bibliographic sources. Secondary analysis and interpretation of the results of sociological surveys, systematization and classification of the theoretical and factual materials used, analysis of management practices and experience of universities in the conditions of extreme transition to remote mode were carried out.

REVIEW AND DISCUSSIONS

In order to support medical education, it has become necessary for medical schools to transit to online learning as the main means of delivering the curriculum. In many countries, the experience of teleworking and studying has shown that the system of higher education faces a number of challenges. Some of them are related to problem areas that existed even before the pandemic, which became more aggravated after its onset and aggravated during martial law. Others are new tasks and points of growth.

Many researchers believe that the educational practices established during the pandemic cannot be referred to as well-planned and built, high-quality online learning. The new phenomenon is called Emergency Remote Teaching and Learning 2 or Emergency Remote Teaching (ERT). ERT is not a full-fledged equivalent of either full-time or distance education [6]. If the goal of online learning is to recreate a complete educational environment, then ERT is a temporary transition to an alternative teaching format due to an emergency.

In the course of the study, 5 main challenges were identified that most universities face, namely: time constraints, emotional tension and stress, communication difficulties and lack of communication, technical and technological problems, and development of practical skills.

CHALLENGES

TIME LIMITS

Due to the need for a quick transition to a remote format, significant difficulties arose with the launch of online training programs in the shortest possible time. Although the process of digital transformation of higher education began several years ago, the pandemic has accelerated it, leading to fundamental changes in a matter of weeks. However, due to the developed state of war in Ukraine, it became necessary not only to continue teaching, but also to work on transferring educational content and materials to the online space, mastering new forms of education, as well as involving and motivating students.

Teachers had to quickly adapt to new online methods, in some cases with little to no training and in record time. This transformation was a hasty and forced circumstance.

The sudden shift to the use of online learning on a large scale led to inconsistencies with curricula [7], which in turn had to be urgently changed to make up for lost hours that were not spent in classrooms, laboratories [8] or at the bedside [9].

The situation was also complicated by the fact that the process of transition to online learning, both in the case of a pandemic and a state of war, did not happen at the beginning, but in the middle of the semester. Time constraints have raised serious concerns about the successful completion of the academic year and guarantees that students would receive their scheduled academic credits. Conducting examinations, as well as planning for the next academic year and next semester, taking into account failures of the academic schedule, plans and procedures, was a major challenge. In some cases, the difficulties in organizing and conducting a distance educational program related to desynchronization of the study group due to the difference in time zones, with some students relocating abroad, or a general lack of time due to increased work and study load.

Remote mode did not reduce the teacher workload as expected by upscaling or reusing teaching material.

On the contrary, the workload has increased due to the need to check, monitor learning progress and give feedback. And this despite the fact that teachers and university staff have already tried to balance teaching, research and official duties, not to mention the balance between work and personal life. In addition, teachers had to prepare and conduct classes from home with all the practical and technical problems associated with this, and often without proper technical support [6].

Degree seekers, as well as educators, have faced difficulties and challenges in adapting to the abrupt and unplanned transition to online learning. To a greater extent, this concerned the problems of independent organization of study time, higher requirements for self-motivation and self-discipline [10], and was also associated with the need to master a variety of skills, competencies and resources in a short time [11].

The sudden increase in workload was mainly due to students increasing their own efforts to catch up with modules without the personal support they previously had from mentors and teaching assistants, proving their involvement to their tutors by completing more assignments than were previously required [12].

It can also be noted that during the period of martial law, for junior students of medical schools, a certain complexity of self-organization was compensated by the relative ease of the material of general educational disciplines, in senior courses, the greater volume and complexity of the educational material was compensated by the already formed learning skill, the ability to use the accumulated and systematize the newly received knowledge.

Although problems with workload at universities are explained by the difficulties of adapting to new study habits [13], they can also be explained by problems with well-being – a state of chronic stress, fear for one's life and the lives of significant others [14].

One of the consequences of the high level of workload was an increased risk of emotional burnout for teachers and students.

EMOTIONAL TENSION AND STRESS

The pandemic, the war and, as a result, forced distancing have affected the emotional, psychological and social well-being of both teachers and degree seekers.

The medical school education period is seen as a critical period of time when education seekers transition from late adolescence to adulthood. This is an extremely sensitive stage of life, gotten associated with emotional problems and mental disorders. In addition, this period is accompanied by a significant increase in risky health behaviors. It is obvious that in a situation of a pandemic, and even more so a state of war, these patterns are exacerbated.

In the world, the problem of the psychological well-being of students is actively studied through such topics as the psychological health and inequality of students, the relationship of physical and mental health with the academic performance of students, mental disorders, suicidal thoughts and related behavior, the impact of anxiety and academic stress on academic success, gambling addiction among students, depression among students, etc.

Stress, anxiety, and anxiety about contracting coronavirus and changing mental health were also noted in a quantitative and qualitative study conducted among public university students in the United States [15].

Several studies highlight the negative impact of homeschooling on the emotional well-being of students not accustomed to distance learning and report several causes and outcomes. For example, students' well-being problems have been found to be positively associated with inappropriate learning environments [13, 14], feeling overworked, being overly concerned about their academic achievements, losing a job [14], career problems [16, 17], concerns about physical health [12].

Students noted a lack of motivation, difficulty concentrating and asking questions. Part of this may be due to the lack of interaction with friends and colleagues, which leads to increased anxiety [7].

In addition, students who already experienced financial problems were more likely to develop depression and anxiety [16], postpone graduations, and change career preferences [17].

The negative psychological consequences of distance learning during the COVID-19 pandemic and exacerbated during martial law cannot be ignored. A significant increase in the volume of homework in many disciplines studied by students against the background of self-isolation, limited space, inactivity, a constant feeling of anxiety and fear for life, leads to a deterioration in psychological and physical health, regardless of the country, level of training and income level. These problems are of great concern and urgent support may be needed to mitigate them.

COMMUNICATION AND LACK OF COMMUNICATION

The transfer of educational process to online environment is associated with a change in communication practices. Oral synchronous communications (classrooms, exams, consultations, informal "live" communication) predominate in classical full-time education. In distance learning, the volume of written asynchronous

communication is increasing (independent study of educational literature and other educational content, completion of written assignments followed by written feedback from teachers). The online format reduces the opportunities for emotional exchange and flexible response that are inherent in face-to-face oral communication.

This inevitably leads to communication losses, such as the lack of immediate response and reactions from students when discussing the topic, as well as limited opportunities to directly address the teacher with a question (during or after class). 64.7% of teachers complained of "discomfort of speech", the feeling of "talking into the void" when lecturing (especially when the cameras are turned off) [18]. The group dynamics inherent in face-to-face classes do not replicate well in an online environment.

Such communication "hindrances" make it difficult to involve students in the learning process, have a negative impact on communication between teachers and students, and reduce its effectiveness.

More than half of students note the lack of face-to-face communication with teachers and fellow students as the main difficulty in organizing distance learning. In many of the studies that have been conducted, students describe feelings of isolation from their instructors, from course content, and from their fellow students.

Separate studies among Norwegian and Malaysian students show that 69.6% of students had a limited opportunity to interact with lecturers, and 62.7% had poor communication with lecturers and other students [19].

In terms of individual barriers, 74.1% of students were not motivated to study online, 71.5% could not study as well as in the classroom, and 58.2% did not agree to take online courses in the future [19].

Video webinars (for example, on the Zoom platform, Meet), as close as possible to the format of face-to-face classes, did not provide the same quality of contact and full-fledged interaction of participants in the educational process as face-to-face classes. Some students find it difficult to adapt to the new format. Almost a third of the students felt embarrassed and uncomfortable when the teacher asked to turn on the webcam. The students name such limiting factors that did not allow them to fully participate in the educational process as poor Internet connection; the session being recorded; not wanting their classmates to be part of their personal space; features of the home environment; appearance, etc. During martial law, constant and prolonged air raids are also a very strong limiting factor, which not only makes it impossible to take part in classes, but also to assimilate the material provided in full. Although, in turn, the students felt that the teachers were mainly engaged with students who had the camera turned on. 35% of students find it difficult to ask questions to a teacher online, they preferred to remain silent [20] and did not want to directly and openly share their views. Some students, using their invisibility, simply avoided answering questions. Also, students had a high threshold barrier for sending questions to the teacher by e-mail.

Most consider online seminars with a teacher via video link to be the most effective form of distance learning. The attendance of such classes is higher. A small number of students called the online lecture format effective [21]. About half of the students preferred lectures "on record", but at the same time, noting that lectures should not last more than 45 minutes [22].

Online classes also place limits on providing feedback, posting follow-up questions during or after class, and asking for clarification on learning issues. Most students believe that the use of online distance learning classes threatens effective communication between group members, especially between those who do not know each other. Some participants also expressed concerns about the difficulty of obtaining basic contact information from their fellow students. Even students who have previously collaborated may find it difficult to meet and not have timely and in-depth communication and discussions, unlike regular classroom sessions. At the same time, communication in social networks does not compensate for "live" communication [23].

Teachers also talk about the lack of effective interaction between students during discussions in online classes. In particular, they do not have strong evidence of whether their students actively participate in virtual discussions. According to the results of separate studies, 81.2% of teachers complained about the difficulty in assessing the degree of attention of students due to the lack of direct eye contact "face to face". A decrease in interaction with students during classes were reported by 65.9% of teachers, and noted the distracting effect of the online environment [18].

Under normal circumstances, as students are in the active learning stage, they seek some awareness or social presence in their learning environment, even in online classrooms. Students need clear social positions and identities in relation to other students. In the conditions of life on campus, they can participate in various activities, actively interact in student communities. University students who interact more with each other have high academic self-efficacy.

Teachers of older generations, theoretically the most experienced and competent in their professional field, turned out to be the least ready to use digital technologies in the educational process, adapt educational and

methodological tools and build communication with students through Internet services, neither during classes nor outside (via e-mail, instant messengers, etc.) [24].

Not all teachers had the skills and competencies of virtual interaction, the ability to work productively and involve others in the work. In online classes, teachers had difficulty engaging students in discussions. As a rule, only 15–20% of those present took part in the discussions [24].

In addition, in distance learning, teachers have encountered the fact that it is impossible to guarantee academic integrity. And proctoring was not very effective in this. With remote testing, it is difficult to solve problems with identification, unauthorized use of textbooks, preventing the use of devices (for example, phone, Bluetooth). In one survey, 73.6% of students found it easier to cheat on online exams than on regular exams. [25]. And this is despite the fact that in medical education, erroneous decisions on exams can have detrimental consequences for patients and high-quality treatment.

All this testifies to the difficulties of changing the formats of learning, verbal and non-verbal communication among the participants in the educational process. Lack of effective communication is one of the biggest limiting forces for organizational educational activities. There is a relationship between the communicative climate and learning productivity. Traditional educational practices, design and components of the educational process (types and forms of training sessions, knowledge testing, etc.) in the online environment are losing their effectiveness and require adaptation.

TECHNICAL AND TECHNOLOGICAL PROBLEMS

The main challenges in adapting to online learning have been technical issues that need to be addressed as they may create an obstacle to the adoption of the online learning system by many students.

While appropriate digital infrastructure and platforms are required for the successful implementation of online learning, poor internet access combined with a lack of laptops and computers has become a major barrier for most students.

For example, according to studies, 66.4% of students in Malaysia [19] and 61% in India faced problems with Internet access. In more developed countries, this percentage was much lower, for instance, 21.5% in the UK, 15% in Finland [7, 26].

In some cases, poor Internet access has led to decisions to suspend or cancel academic activities, and has also made it difficult to provide students with equal learning opportunities, even in middle- and high-income countries [27].

Not all students have devices that provide the full range of features needed in the learning process. The vast majority of students own the electronic devices required for online learning (97%) and are willing to invest in such devices (74%). The majority of participants preferred a computer (79%), a smaller number preferred a tablet (20%), and only a very few used a smartphone (1%) [28].

Despite this, a significant part of the students still experience problems with the acquisition of the necessary technical means. For example, according to one study, 38.6% of students agreed with the difficulties in purchasing a computer or laptop [19]. This is especially true for families with two or more children, whose financial resources are limited. Often, students did not have enough work space at home to organize their studies.

The inequality of individual technical and everyday capabilities of the main participants in the educational process, i.e. teachers and students, depending on which their access to educational resources and the quality of the educational process turned out to be, was aggravated by the inequality of the technical capabilities of the universities themselves. Even universities in countries with good internet connectivity report financial difficulties in investing in tools and online licenses [27].

Based on this, distance learning may not reduce, as expected, but rather increase the digital gap.

Moreover, students with fewer opportunities (for example, lack of digital or inappropriate equipment, lack of Internet or slow connection) and poor digital skills are likely to be more affected by online learning, which will increase existing inequalities [29].

In addition, the transition to online learning has probably exacerbated the inequality between universities with different levels of development of educational technologies.

The technologies most commonly used by universities to support online learning during lockdown are the university web platform; instant messaging tools (WhatsApp, Telegram); video conferencing tools (Zoom, Skype, Google Hangouts, Google Meet); educational applications (Google Classroom); as well as e-mail and telephone conversations to maintain individual contact with students. The most widely used platforms are GoToMeeting, Skype and Zoom [30]. In general, other technologies (CiscoWebEx, GoToMeeting, Microsoft Teams, Monosnap, Loom, OBS, etc.) have also proved to be useful.

It can be assumed that the most commonly used educational technologies are tools for synchronous collaboration with a high potential for simulating face-

to-face communication. This is especially true for video conferencing. Most likely, this can be explained by the desire to recreate the situations of communication and interaction that occur during face-to-face classes on campus.

But the solutions used were not originally intended for educational purposes and, in the process of application, created additional problems in turn.

For example, the introduction of such software as Zoom and Microsoft Teams has exposed many privacy and data security issues. Some of this software collects and uses data, tracks meetings, sometimes without the knowledge of participants, and uses this data to provide personalized ads to users. In this regard, some universities announced the abandonment of the Zoom platform and the transition to GoogleMeet or other applications. There was such a phenomenon as Zoom bombing, which characterizes actions related to the violation of online spaces, including hacking virtual classrooms [31].

Digital education requires universities to have the appropriate infrastructure and technological platforms, reliable servers that can withstand virtual workloads, and methodological training of teachers and students for online delivery using all available technical and educational resources.

Work in remote mode showed a high degree of differentiation of universities in terms of the level of adaptation to pandemic realities and the realities of martial law and the danger of stratification of universities according to the degree of their involvement in distance education.

Before starting to work remotely, most higher education institutions did not have the infrastructure to deploy full-fledged distance learning. There was no high-speed access to the Internet and there were no specialized data storage systems to accommodate information systems. The load on university websites, information systems and databases has increased significantly, which has led to instability and failures in their work.

Learning management systems (LMS) (for example, Blackboard, Moodle), which were already used in most higher education institutions, facilitated attendance, student registration and content distribution, testing and assessment management, but were practically not used to organize full-fledged virtual classes, administration or monitoring [32].

It should be noted that some university professors have already used distance formats for teaching students in their practice. This to some extent facilitated the transition to new working conditions. The results of separate studies showed that the majority of teachers (70%) were ready to choose online classes for curriculum management [33]. In turn, 64.2% of educators cited technical issues such as poor connectivity, power outages, broadband issues, and poor audio and video quality as the main disadvantages of online classes, indicating that they do not prefer online learning [34].

Compared to classroom learning, online learning requires higher basic computer skills, such as good knowledge of computer technology, proper handling of various teaching and learning tools, knowledge of available platforms and services for remote learning, their functionality, and the need to quickly solve specific problems during training sessions [35].

Thus, the general level of digital literacy of students, the availability of communication skills in social networks can become the basis that will allow students, with appropriate technical capabilities, motivation and assistance from teachers, to quickly master distance learning technologies, which will positively affect both the attitude of students to these technologies, as well as the quality of education.

PRACTICAL SKILLS

Medicine is a discipline with a high degree of practicality

Despite the high demand for online learning among medical students, there are parts of medical education that seem inappropriate for online learning.

Medical education differs from other types of education in that it is not possible to fully master practical and clinical skills in a distance form, and training is traditionally carried out full-time.

Face-to-face clinical education, with its dynamic interaction between students, patients, and doctors, contributes to the formation of the necessary clinical skills and professional behavior [36], which cannot be formed by online distance learning.

Clinical practice is a significant part of the training of future doctors, the most important exams are also taken in clinics. The inability to attend medical institutions has called into question both the practical training of students and their official certification, which can negatively affect employment prospects [37].

Acquiring clinical skills remains a significant barrier to online learning. For example, studies conducted in the UK and Germany show that 75.99% of medical students believe that online education has not been able to successfully replace the clinical education they received through direct contact with the patient, while 82.17% reported that they cannot learn practical clinical skills through the online learning mode. 91% of students indicated that they would like to continue their education with real patients [7, 21].

Under conditions of forced distancing, the only real opportunity for medical students to gain practical experience is voluntary visits to medical institutions. However, it does not provide knowledge and skills beyond a particular specialization [38].

The specificity of medical education is such that most of the clinical competencies of the future doctor being formed are inextricably linked with forms of training that require personal presence, these are, first of all, communication skills and practical manipulations. In addition, teamwork is fundamental to health care. Removing face-to-face clinical rotations from the training program potentially threatens the development of key teamwork skills.

At the stages of clinical medical education, there is a greater need for experimental and clinical practice than at the stages of general and basic medical education [39]. Practical skills acquire a complex structure and require not a mechanical demonstration of the skill, but, to a greater extent, the ability to recognize, interpret, and analyze the data of the clinical situation. Visualization plays a key role in the acquisition of knowledge by the future doctor. It is impossible to imagine the study of anatomy without working in dissection, histology without working with microspecimens, surgery without monitoring operations, etc.

Through observation, practice, and repetition under the guidance of experienced clinicians, medical students also acquire the necessary clinical skills for patient care, which are an integral part of a doctor's daily practice.

Building relationships with the patient, the use of patient-oriented communication skills correlates with the effectiveness of the doctor's professional activities, psycho-emotional well-being and job satisfaction, and also affects the patient's well-being and health.

In addition, despite the positive effect of internships, in a number of cases, a student who is faced with the realities of his future specialty has negative emotions, frustration, a decrease in the level of motivation up to refusal to study in this program. At this stage, it is very important to help the student adapt, reduce stress levels and stabilize psycho-emotional perception. Therefore, human interaction is extremely important for medical practice [40].

In turn, the lack of tactile awareness and practical instruction can hinder the learning of complex practical skills [1].

CONCLUSIONS

The situation with the pandemic, and subsequently with martial law, developed so quickly and was so unprecedented that no one had the opportunity to use someone else's experience to overcome the problems associated with the need for self-isolation. Universities from different countries, regions and cities had to make their own decisions about the conduct of the educational process. Therefore, it is necessary to compare and analyze the world and our own experience in conducting distance learning in order to develop an individual strategy for universities on this issue.

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CASE STUDY

THROMBOTIC MICROANGIOPATHY: DIAGNOSTIC CHALLENGES IN THE PRIMARY MULTIPLE NEOPLASM INVOLVEMENT WITH PREVALENT METASTASIS WITH GRANULOMATOSIS INFLAMMATORY FOCUSES

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ABSTRACT

Patient, who died during the hospital stay, had hemoblastosis and syphilis in the reported medical history. While the patient was examined doctors suspected the presence of malignancy with unknown primary localization with multiple metastatic injuries with clinical and laboratory TTP signs (hemorrhagic syndrome, thrombocytopenia, shystocytosis, and non-immune hemolytic anemia). Despite treatment, the general patient's condition progressively worsens with increasing multiple organ decompensation signs. In the final stage of the disease course, after heart arrest and the appearance of clinical death signs CPR measures were performed according to complete guidance, but CRP had no positive effect. Biological death was constated.

Considering the criteria of the diagnostic clinical and laboratory dyad (thrombocytopenia and microangiopathic hemolytic anemia), the data of the pathological examination (multiple metastatic lesions, inflammatory process, tumor intoxication, thrombosis), the combination of manifestations of chronic myeloid leukemia, prostate cancer with multiple metastases, tertiary syphilis served as a condition for the initiation of TTP, which was of decisive importance in the development of the patient's death.

KEY WORDS: thrombotic thrombocytopenic purpura, thrombotic microangiopathy, plasma exchange therapy, ADAMTS13, von Willebrand factor

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INTRODUCTION

Thrombotic thrombocytopenic purpura (Moschcowitz syndrome, TTP) is a minor vessels injury (microangiopathy) that is characterized by hemolytic anemia, intravascular coagulation, thrombocytopenia, injury of kidneys and nervous system [1 - 3]. According to the Chiasakul, T. & Cuker A. [4] is a rare, life-threatening disease with an incidence of approximately 2 persons per million per year. TTP has an extremely aggressive course and requires starting therapy as soon as possible (during first hours after onset). In the absence of adequate and urgent management, the lethality is high and lies at 72-94% [5]. That is why in the absence of other possible reasons for thrombocytopenia and non-immune hemolytic anemia TTP should be thought as a proved diagnosis and treatment should be started. Early adequate therapy allows to avoid life-threatening key-organ injury [6, 7].

CASE REPORT

Patient G., a 54-year-old man born in 1965, was admitted on the 15th of October 2019 to the hematological clinic of the National Military Medical Clinical Centre "Main Military Clinical Hospital" with complaints of non-severe fatigue, intermitted petechial skin rash and excessive bleeding after small injuries. He reported that 30 years ago, he had been admitted to the dermatological department for syphilitic treatment. After receiving primary therapy and leaving the dermatological department, he never checked again for the effectiveness of syphilitic treatment and was not examined by a dermatologist.

While admitted in October 2019, a general patient's condition was not bad. In the blood count test (BCT), severe anemia (RBC – 1,12×1012/l, MCV – 103 fl., hemoglobin (Hb) – 78,0 g/p), reticulocytotic changes in the blood – 45%) and thrombocytopenia (25,0×109/l) were

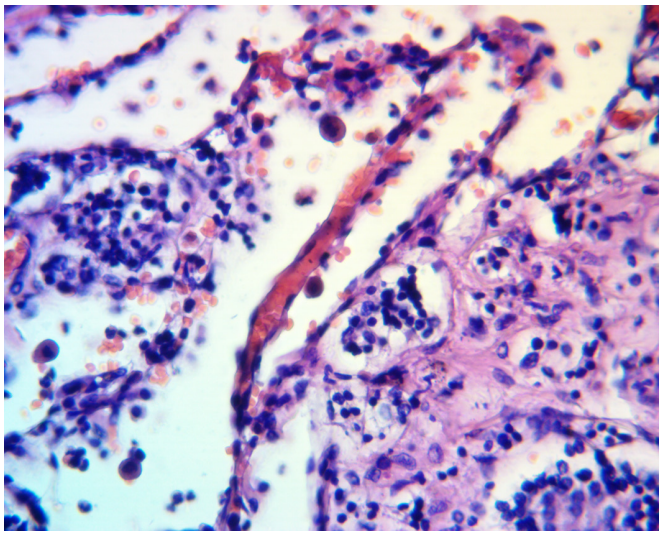

Fig. 1. Red clots in pulmonary vessel in the stage of erythrocytes agglutination (with hematoxylin and eosin, enlargement ×400).

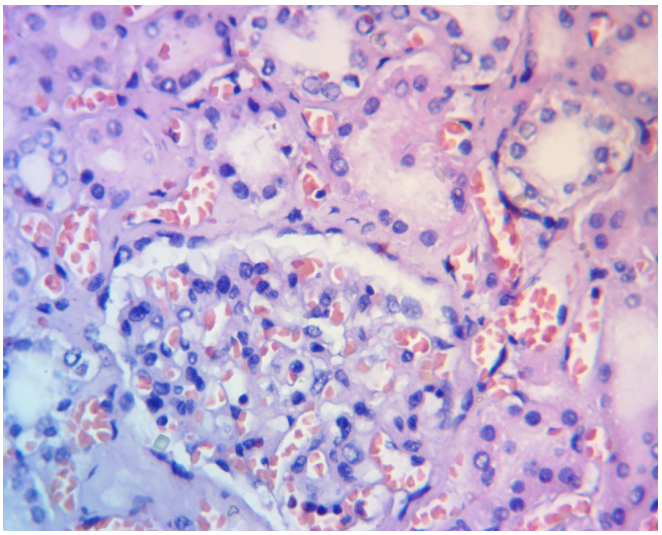

Fig. 2. Red clots in small vessels of kidney glomeruli in the stage of erythrocytes agglutination (with hematoxylin and eosin, enlargement ×400).

detected. While checking RBC morphology, shystocytosis was detected. Direct and indirect Coombs tests were negative. Myelogram reported that red bone marrow (RBM) had a few numbers of cells; megakaryocytes were absent; thrombocytes were in a small amount in the microscopic unit of view. Leuco-/erythrocytes ratio was increased up to 4.88:1 (normal range 3:1–4:1). Myeloproliferative process was suspected after performing of cytomorphological, cytochemical immunophenotype blood and RBM examining.

Chest X-ray did not report any infiltrative or volume findings in the lung parenchyma. US-findings: liver and spleen were enlarged, as well pleural effusion on the right part of the chest was observed. Finally, we received positive test results on the presence of antibodies against Treponema pallidum.

In the period of 15-23 of October 2019, the patient's general condition was quite good, but on October 24 state rapidly became worse: respiratory deficiency appeared and still progressed (dyspnoea at rest which increased with minimal physical activity). Myelogram results (October 24) reported the presence of myeloproliferative disease and recommended performing a trepan biopsy, but the last examination procedure was unavailable due to severe thrombocytopenia.

Multidetector computed tomography of the brain, chest, abdomen and pelvis helped to visualize such changes: signs of multiple segmentary bilateral pneumonia; bilateral hydrothorax (35 mm in the right and 23 mm in the left pleural spaces); hydropericardium (up to 33 mm); mediastinal lymphadenopathy (maximal sizes up to 16-19 mm); hepatosplenomegaly; rectoabdominal lymphadenopathy (maximal sizes up to 25-28 mm); ascites; generalized multiple skeletal bone involvement.

The patient was transported to the intensive care unit (ICU) as his condition rapidly worsened with pneumonia, respiratory insufficiency, bycytopenia, and severe intoxication. In the ICU patient's condition constantly was getting worse with the progression of multiple organ insufficiency. The dermatologist examined the patient, and his diagnosis was: Lues latens (latent syphilis).

On the evening of October 24th (from 5 p.m. to 11 p.m.), moderate ecchymosis appeared on the skin of the abdomen, legs and arms. Respiratory insufficiency had progressed, the patient became hemodynamically unstable, and anuria appeared, as a result, a heart arrest happened at 23:27. Cardio-pulmonary resuscitation CPR was ineffective. Biological death was constated at 23:57 on October 24th, 2019.

The pathological expertise of the body detected some macroscopic changes that could be divided into several groups:

- hemorrhage syndrome: petechial and ecchymosed hemorrhages in the skin, on the parietal pleura, on the visceral layer's pericardium, and in the epicardial adipose tissue.
- granulomatous inflammation: typical hilly layering on the diaphragmatic surface and aortic intima.
- possible (or probable) primary malignancy: subcapsular prostatic overgrowth of yellow tissue bilaterally with over 50% of prostatic involvement.
- possible (or probable) metastatic grey-yellowish injury of subpleural layers of the lung parenchyma, lymphatic nodules, and bone tissue (Fig. 1).

Further histological examination of deceased material helped us to find signs of the following diseases:

- chronic myeloid leukemia (CML)
- moderate- and low-grade differentiated acinar pros-

tatic adenocarcinoma with metastasis in pelvic and paraaortic lymphatic nodules, lung parenchyma, and skeletal bones
- tertiary syphilis with granulomatous inflammation of the diaphragm and syphilitic gumma and mesaortitis
- generalized thrombotic microangiopathy was observed in the vessels of the lungs, kidneys, prostate, and spleen.

Patient G., who died during the hospital stay, had hemoblastosis and syphilis in the reported medical history. While the patient was examined doctors suspected the presence of malignancy with unknown primary localization with multiple metastatic injuries with clinical and laboratory TTP signs (hemorrhagic syndrome, thrombocytopenia, shystocytosis, and non-immune hemolytic anemia).

Despite treatment, the general patient's condition progressively worsens with increasing multiple organ decompensation signs. In the final stage of the disease course, after heart arrest and the appearance of clinical death signs CPR measures were performed according to complete guidance, but CRP had no positive effect. Biological death was constated.

From the clinician's point of view, in this case, the rapid course of the disease was caused by the onset of generalized thrombotic microangiopathy, which was a sign of probable TTP that was the cause of the appearance of multiorgan decompensation and sudden death (Fig. 2.).

Based on pathological data from the deceased patient's body we defined that the main diseases were chronic myeloid leukemia and prostatic cancer with multiple metastases. The most important complications that caused death were named such conditions: generalized thrombocytic microangiopathy and bilateral pneumonia because of malignancy intoxication which directly caused the patient's death. As an important comorbidity tertial syphilis with diaphragmed gamma and syphilitic mesaortitis.

Atypical disease courses, anemia and thrombocytopenia, kidney injury, and TTP do not allow us to manage diagnostic procedures for full vital diagnostics of prostatic malignancy with metastases. The only pathological expertise with microscopic histological examination of deceased tissues defined multiple skeletal bone, lymphatic nodular, lungs metastases with thrombosis of small vessels in lungs, kidneys, prostatic gland, and spleen.

Analyzing this clinical case, we should mention with a high level of probability that TTP was a crucial process in pathology.

The leading role in the pathogenesis of TTP was defined as a deficiency of ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin-1-like domains) – metalloprotease from the family of peptidase proteins ADAM. They have a specific biological role which includes the degradation of extracellular domain transmembrane proteins. Predisposition to the occurrence of TTP is associated with pregnancy, HIV, autoimmune and inflammatory processes, transplantation, and tumors [8].

Intoxication occurs at a particular stage of the course of tumor diseases because of insufficient blood supply and oxygenation, subsequent necrosis of atypical cells and entry of decay products into the bloodstream, changes in blood rheology, and disturbances in the hemostasis system with the occurrence of thrombosis. In this case, there was thrombosis of small blood vessels, which most likely could be caused, on the one hand, by direct tumor damage to the bone marrow by atypical cells in chronic myelogenous leukemia and prostate cancer and, on the other hand, by the mediated systemic effect of tumor tissue decay products.

Another mechanism of the possible development of TTP is the inflammatory process, the sources of which in this deceased could be hematogenous metastasis and phenomena of tertiary syphilis. Another source of inflammation can be humous infiltrates of the diaphragm and mesaortitis, which existed in patient G. for many years.

TTP is based on damage to the endothelium of small vessels with subendothelial deposition of fibrin, followed by aggregation of platelets and partial or complete occlusion of vessels (thrombotic microangiopathy). First of all, the brain, kidneys, and lungs are affected. Narrowing the blood vessels' lumen contributes to the mechanical destruction of erythrocytes with the development of microangiopathic hemolytic anemia (MAHA). The fragmentation of erythrocytes characterizes it after wrapping them with fibrin threads under the influence of blood flow. The attachment of erythrocytes wrapped in fibrin threads to the endothelium of vessels occurs after the interaction of erythrocyte integrin and vascular cell adhesion molecule (VCAM-1) [9]. Another mechanism of erythrocyte attachment to vascular endothelium involves the interaction of large multimers of the Willebrand factor (Wf) as a «joint» between integrins present in the membranes of both young erythrocytes and endothelial cells.

Decreased activity of ADAMTS13 is found in disseminated intravascular coagulation syndrome (DIC), cirrhosis, uremia, acute inflammatory diseases, and in the postoperative period [10, 11].

The difficulty of diagnosing Moshkowitz's disease is explained by the absence of specific clinical symptoms in patients. The prodromal period is characterized by weakness, exhaustion, and lack of appetite. The disease develops, as a rule, acutely against the background of complete health. Often there is a prodrome resembling an acute respiratory syndrome, then a comprehensive clinic appears. Sometimes this is preceded by infectious diseases and drug intolerance. The first signs may be a

weakness, headache, dizziness, nausea, vomiting, and abdominal pain.

Classic pentad is typical for TTP [12]. It includes**:**

1) hemorrhagic syndrome (hemorrhages on the skin, nose, bleeding gums, menorrhagia, and others) on the background of thrombocytopenia (often severe with a platelet count < $30{,}0\times10^9$/l);
2) microangiopathic hemolytic anemia (MAHA), which is manifested by reticulocytosis, the presence of fragmented erythrocytes (schistocytes)) in a blood smear, hyperbilirubinemia, a negative direct antiglobulin test;
3) neurological disorders (consciousness up to coma, headache, convulsions, focal disorders (hemiplegia, visual impairment, aphasia, paresis);
4) kidney damage: microhematuria and proteinuria (most characteristic), cylindric particles in urinalysis, increased creatinine (about half of patients);
5) fever.

TTP can manifest such diseases: as pancreatitis, hepatitis, rhabdomyolysis, acute respiratory distress syndrome, myocardial infarction, nonocclusive mesenteric ischemia, peripheral ischemic syndrome, and skin gangrene. A significant part (35%) of patients with TTP develop abdominal syndrome (severe abdominal pain, nausea, vomiting) due to abdominal ischemia.

Determination of the level and activity of ADAMTS13 is not yet a routine procedure in hematological practice (in domestic laboratories the test is not performed at all) [3], that is why primary diagnostic criteria were proposed [13], the combination of which (thrombocytopenia and MAHA (dyad) in the absence of other established causes of the disease is sufficient to establish a diagnosis of TTP.

The determination of the signs of the disease that are minimally sufficient for the diagnosis of TTP led to an increase in the number of detected patients without all the signs of the classical pentad (the frequency of the occurrence of the pentad 34 – 77%). Due to this, therapy begins to shift to the first days of the disease. Patients are usually hospitalized: in the surgical department (abdominal pain with suspicion of «acute abdomen»); infectious (jaundice and fever), as well as in intensive care, neurological, therapeutic, and gynecological departments. Diagnosis of TTP, based on two criteria, requires the exclusion of diseases in which hemolytic anemia and thrombocytopenia may occur. In the presence of reticulocytosis and normocytic, laboratory doctors paid attention to the presence of schistocytes (fragmented erythrocytes).

Examination of the general blood count test and analyses of the morphology of erythrocytes, a negative direct antiglobulin test (DAT), and anamnesis allows us to quickly exclude such conditions recommended for differential diagnosis as: megaloblastic anemia, paroxysmal nocturnal hematuria malignant arterial hypertension, Evans syndrome (combination of immune thrombocytopenia and anemia), lymphoproliferative diseases.

Differential diagnoses with infectious diseases leading to bacterial septicemia or systemic inflammatory response syndrome are more complicated. Bacterial (*E. coli* 0157: H7) and viral (HIV, CMV) infection can be the cause of TTP [14, 15]. Sepsis can be manifested (especially when DIC syndrome occurs) by thrombocytopenia with a hemorrhagic syndrome, hemolysis with the presence of schistocytes and multiple organ failure. To clarify the diagnosis, it is necessary: blood cultures, search for the focus of infection, and assess the level of procalcitonin, a C-reactive protein (CRP).

Diffuse connective tissue diseases can clinically have a picture similar to TTP: thrombocytopenia, hemolysis, sometimes in combination with fever and damage to the central nervous system and kidneys. First of all, this concerns SLE, antiphospholipid syndrome [16]. SLE is characterized by: hemolytic anemia with a positive Coombs test, the presence of LE cells, and antinuclear antibodies. However, it should be remembered that TTP can be a secondary, that is, a diffuse complication of a connective tissue disease.

It is noted that diseases that occur with schistocytes and thrombocytopenia, symptoms similar to TTP can have disseminated intravascular coagulation syndrome (DIC syndrome) and hemolytic uremic syndrome (HUS also called Gasser syndrome). DIC is therefore called a syndrome that is secondary to the severe course of another, more often already established disease, and is accompanied by corresponding changes in the hemogram. HUS characterized by a combination of thrombotic microangiopathy with hemolytic anemia and acute renal failure. HUS is sometimes manifested by multiorgan pathology, including neurological complications, liver and heart injury. Clinical symptoms of HUS, significantly when the nervous system is affected, differ little from TTP, their differentiation is difficult [17]. Some experts consider TTP and HUS as one disease - TTP-HUS [18], however, with «classic» HUS, the activity of the ADAMTS13 enzyme is normal [3].

Thus, considering the criteria of the diagnostic clinical and laboratory dyad (thrombocytopenia and MAHA), the data of the pathological examination (multiple metastatic lesions, inflammatory process, tumor intoxication, thrombosis), the combination of manifestations of CML, prostate cancer with multiple metastases, tertiary syphilis served as a condition for the initiation of TTP, which was of decisive importance in the development of the patient's death.

CONCLUSIONS

TTP is life-threatening disease which does not have any specific symptoms.

In all cases of the firstly detected thrombocytopenia, it is necessary to evaluate the possibility of TTP by the pentad or dyad symptoms to decide whether it is non-immune and obligatory to focus on morphology of the erythrocytes to exclude shystocytosis.

In our case, the main reasons for the development of thrombotic icroangiopathy can be the presence of two tumor diseases - chronic myeloid leukemia and a tumor lesion of the prostate with widespread metastasis and the presence of foci of granulomatous inflammation

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