

ORIGINAL ARTICLE

COMPARATIVE ANALYSIS OF FRACTAL DIMENSIONS OF HUMAN CEREBELLUM: IMPACT OF IMAGE PREPROCESSING AND FRACTAL ANALYSIS METHODS

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Nataliia I. Maryenko, Oleksandr Yu. Stepanenko
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

The aim: To compare the values of the fractal dimensions of human cerebellum obtained using different algorithms of image preprocessing and different methods of fractal analysis.

Materials and methods: The study involved 120 people without structural changes in the brain (age 18-86 years, 55 men and 65 women). T1- and T2-weighted MR brain images were studied. Fractal analysis was performed using box counting and pixel dilatation methods. Fractal dimensions of cerebellar tissue as a whole, cerebellar cortex and its individual layers, cerebellar white matter were measured and compared to each other and to fractal dimension of cerebellar white matter determined in cadaveric cerebella.

Results: It was no significant difference between fractal dimension values of cerebellar tissue as a whole measured on T1 and T2 weighted magnetic resonance images of cerebellum, and fractal dimension values measured on the same images using different methods of fractal analysis – pixel dilatation and box counting. T2 weighted images are preferable for fractal analysis of different components of cerebellar tissue. Segmentation according to pixel luminance is the preferable image preprocessing method for fractal analysis of cerebellar cortex as a whole, individual cortical layers and cerebellar tissue as a whole; skeletonizing of cerebellar magnetic resonance images is the preferable method of the image preprocessing for fractal analysis of cerebellar white matter.

Conclusions: The algorithm of image preprocessing, magnetic resonance imaging sequence and method of fractal analysis should be chosen according to aim of quantitative study of cerebellar magnetic resonance images and features of the studied structure of cerebellum.

KEY WORDS: cerebellum, fractal analysis, fractal dimension, magnetic resonance imaging, neuroimaging

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INTRODUCTION

In recent years, fractal analysis is increasingly used in morphological investigations of fractal biological structures. Fractal analysis provides quantitative and objective determination of the spatial complexity degree of different structures of human organism [1-3].

Spatial configuration of different structures of human cerebellum has fractal properties. Cerebellar white matter has a sophisticated tree-like branching pattern. It may be considered as fractal structure as well as cerebellar cortex, which forms a three-dimensional convoluted foliated structure, duplicating external contour of white matter [4]. Fractal analysis is one of the main morphometric techniques that may provide a quantitative morphological assessment of cerebellum [5-9].

For fractal analysis it is necessary to clearly define the boundaries of the studied structure. In view of this, the true fractal dimension of some cerebellar structures can be determined only via study of the anatomical sections of cerebellum, because neuroimaging methods may not have sufficient resolution to clearly define the boundaries of cerebellum and the boundaries between different components of cerebellar tissue. In our previous study, we determined the fractal dimension of white matter on the midsagittal sections of cadaveric cerebella [10]. In further studies we

determined the fractal dimensions of cerebellum and its individual components (white matter and cortex) on the magnetic resonance (MR) images [11-13]; but we faced the problem of adapting these results to clinical practice to quantify MR brain images.

Different image preprocessing algorithms and various fractal analysis methods were used in different studies. The box counting method was applied in the studies of Akar E. at al. [5-7] and in the study of Wu Y.T. et al. [8]; the pixel dilatation modification was applied in the study of Liu J.Z. at al. [9]. In our previous studies we applied both methods: box counting [10, 11] and pixel dilatation [12, 13].

Therefore, to determine the best algorithm for fractal analysis of cerebellar MR images, we decided to compare the true FD values measured in cadaveric cerebella [10] and FD values measured in T1- and T2-weighted MR brain images, with different image preprocessing algorithms and using different fractal analysis methods [11-13]. The present study is a continuation and summarizing of our previous research on fractal analysis of human cerebellum [10-13].

THE AIM

The aim of the study was to compare the values of human cerebellum fractal dimensions obtained using different

algorithms of image preprocessing and different methods of fractal analysis.

MATERIALS AND METHODS

The study involved 120 people without structural changes in the brain (age 18-86 years, 55 men and 65 women). All participants provided written informed consent.

The conclusion of the Commission on Ethics and Bioethics of Kharkiv National Medical University confirms that the study was conducted in compliance with human rights, in accordance with current legislation in Ukraine, meets international ethical requirements and does not violate ethical standards in science and standards of biomedical research (minutes of the meeting of the Commission on Ethics and Bioethics of KhNMU №10 from 07.11.2018).

T1- and T2-weighted MR brain images were studied. MRI was performed on a 1.5 T MRI machine. The image parameters included the following. T1-weighted images: TE (echo time) was 14 ms, TR (repetition time) was 500 ms; section thickness was 5 mm; T2-weighted images: TE was 122 ms, TR was 4520 ms; section thickness was 5 mm. Sagittal MRI projection was chosen for the study (Fig. 1).

Initial preprocessing included segmentation of images. A 2 × 2-inch (128 × 128-pixels) fragments containing the midsagittal sections of the cerebella were copied from the digital magnetic resonance (MR) images (Fig. 1, A, D). The fragments of MR images were segmented using the Adobe Photoshop CS5 software. The structures surrounding the cerebella were initially removed from the images (Fig. 1, B, E), and the pixels in these areas were colored black (T1-weighted images, luminance value of 0 – Fig. 1, B) or white (T2-weighted images, luminance value of 255 – Fig. 1, E). Segmentation was performed according to the pixels' luminance value using the "threshold" tool. The images were segmented into two components: the studied structure (colored white in T1-weighted images or colored black in T2-weighted images) and background (colored black in T1-weighted images or colored white in T2-weighted images). An empirical luminance threshold value of 100 was used for segmentation of T1- and T2-weighted MR images; it revealed the cerebellar tissue as a whole without segmentation into individual components (Fig. 1, C, F).

Fractal analysis was performed using two different methods: pixel dilatation method in the author's modification [14] and box counting method with the Image J software [15]; two-dimensional fractal dimensions (2D FD) were determined [11-13].

Initially, fractal analysis of cerebellar tissue as a whole was carried out using pixel dilatation method. Two fractal dimensions were measured: FD of T1-weighted images (FD(1)) and FD of T2-weighted images (FD(2)). T2-weighted images were more heterogeneous than T1-weighted images, thus, the T2-weighted images were chosen for the study of individual components of cerebellar tissue.

For the further investigations, we selected T2-weighted MR brain images of 30 young adults (18-30 years age range,

15 men and 15 women) among the MR images of 120 persons enrolled in the study. FD values were measured on the same MR images using two different methods of fractal analysis: pixel dilatation method (FD(3)) and box counting method (FD(4)).

Afterwards, the studied MR images were additionally segmented into individual components of cerebellar tissue (Fig. 2, A-E). For that purpose, the "threshold" tool was used. Fractal analysis of individual components of cerebellar tissue was carried out using the pixel dilatation method. We determined FD of cerebellar cortex as a whole (FD(5)), FD of granular layer of cerebellar cortex (FD(6)), FD of molecular layer of cerebellar cortex (FD(7)) and FD of cerebellar white matter (FD(8)).

After the initial segmentation, the image skeletonizing procedure was performed (Fig. 2, F). We used the "skeletonize" tool of Image J software. This tool revealed the main branches of the cerebellar white matter. FD of skeletonized images (FD(9)) was determined using a box counting method.

The obtained FD values were compared with each other and were compared to the FD values of cerebellar white matter obtained in our previous study of cadaveric cerebella [10] (FD(10), FD(11)). The study [10] was carried out on cadaveric specimens: 100 cerebella of people of both sexes who died from causes not related to brain diseases (62 male and 38 female; age range of 20-95 years). Cerebella were obtained during forensic autopsies. The macrophotographs of the midsagittal sections of cerebellar vermis were studied, the box counting method was utilized for fractal analysis; the counting was manual due to impossibility of the automatic image segmentation which is necessary for the automatic box counting with Image J software. The true values of FD of cerebellar white matter were determined (FD(10)). We additionally selected 14 cadaveric cerebella (20-30 years age range) for the present study and calculated FD value of the cerebellar white matter of those objects (FD(11)) to compare with FD values measured on 30 MR images of young persons (18-30 years age range).

A statistical data processing was performed using Excel 2010 software. The following values were calculated: the sample mean (M) and the standard error of the mean (m), the median value (Me, percentile 50) with interquartile ranges (the values of percentiles 25 and 75), the minimum (min) and the maximum (max) values. The significance of statistical differences between the FD values was assessed using the Kruskal-Wallis H test with Bonferroni adjustment for multiple comparisons. The significance level for all results was accepted as $p < 0.05$.

RESULTS

We analyzed FD values of different structures of cerebellum, obtained using different image preprocessing algorithms and different methods of fractal analysis. The obtained values of the analyzed fractal dimensions of human cerebellum are listed in Table I and the distribution of the FD values is shown in Fig. 3. The statistical significance

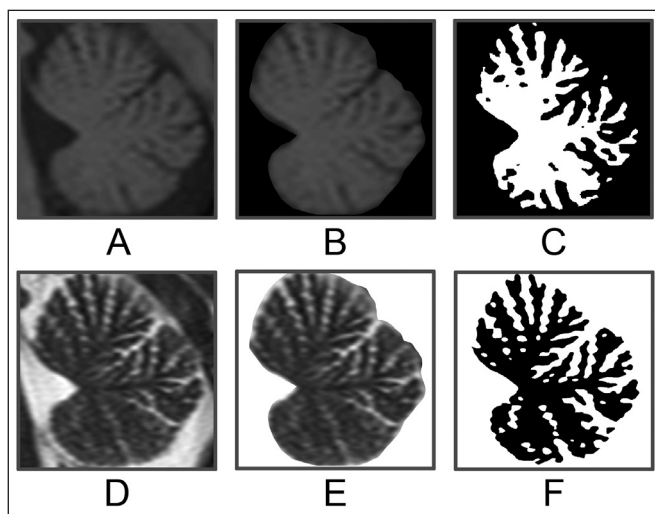


Fig. 1. Pre-processing of cerebellar MR images: segmentation of T1-weighted images (A, B, C) and T2-weighted images (D, E, F).

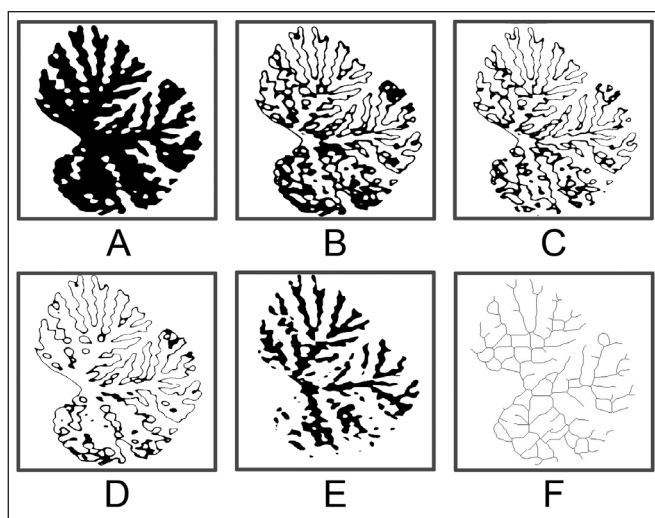


Fig. 2. Pre-processing of T2-weighted MR images of cerebellum. A-E – image segmentation using “threshold” tool: A – cerebellar tissue as a whole (threshold 100), B – cortex as a whole (difference between thresholds 100 and 80), C – granular layer of cerebellar cortex (difference between thresholds 90 and 80), D – molecular layer of cerebellar cortex (difference between thresholds 100 and 90), E – white matter (threshold 80). F – image skeletonizing using “skeletonize” tool: skeleton of white matter.

of the difference between FD values was assessed and null hypothesis was rejected; the difference between the mean ranks of compared FD values was statistically significant. Thus, the multiple paired comparisons between different FD values were provided.

It was no significant difference between FD values of cerebellar tissue as a whole, including FD(1) value measured on T1 weighted images using pixel dilatation method, FD(2) and FD(3) values measured on T2 weighted images using pixel dilatation method and FD(4) value measured on T2 weighted images using box counting method.

FD(1) and FD(2) values were measured on the MR images of the same persons, using the same image preprocessing and the same fractal analysis method (pixel dilatation), but

different MRI sequences were utilized to obtain the studied MR images. The FD values determined on T1 and T2 weighted images were not significantly different and had close comparable parameters of statistical distribution. Therefore, T1 and T2-weighted MR images may be used for fractal analysis.

FD(3) and FD(4) values were measured on the T2-weighted MR images of the same persons and with the same image preprocessing, but different fractal analysis methods were applied. The FD values determined utilizing different methods of fractal analysis (FD(3) – pixel dilatation, FD(4) – box counting) coincided and were not significantly different. This indicates that both methods of fractal analysis may be used to determine FD values of cerebellar tissue as a whole.

All FD values of cerebellar tissue as a whole (FD(1-4)) were significantly different from the FD values of individual components of cerebellar tissue: FD(5-8), FD(9) (FD of skeletonized images) and FD of cerebellar white matter measured on cadaveric material (FD(10-11)). There was significant difference between FD(5) value (cortex) and all other FD values, but there was no significant difference between values of FD(6) (molecular layer of cortex), FD(7) (granular layer of cortex) and FD(8) (white matter) compared to each other.

The FD values corresponding to cerebellar white matter were measured on the same T2-weighted MR images of the same persons, but with different image preprocessing: FD(8) – segmentation with a threshold of 80 and FD(9) – image skeletonizing. The FD(8) and FD(9) values were not significantly different.

The FD values were determined on the different materials (MR images and cadaveric material). FD values of cerebellar white matter measured on the MR images (FD(8) and FD(9)), were compared to FD values of white matter measured on the midsagittal sections of cadaveric cerebella. There was no significant difference between FD(8) and FD(10). But there was a significant difference between FD(9) and FD(10) ($p < 0.05$). This may be caused by difference in the age range: 18-30 years for FD(9) and 20-95 years for FD(10). In our previous study, it was established that FD of cerebellar white matter had a significant strong negative correlation relationship with age ($r = -0.917$, $p < 0.001$). According to this fact, we selected 14 cadaveric specimens (among 100) in the compatible age range (20-30 years) and calculated FD(11). There was no significant difference between FD(9) and FD(11). FD(9) and FD(11) values coincided and had close comparable parameters of statistical distribution and variance of the values. FD(8) values have a much larger variance compared to FD(9) and FD(11) values; this parameter may not be as accurate as the FD of skeletonized images (FD(9)). Thus, FD(9) (measured on skeletonized MR images) may be considered as a best parameter that corresponds to the true fractal dimension of the cerebellar white matter (FD(11)) which can be only measured by direct study of the anatomical sections of cadaveric cerebella. Skeletonizing of the cerebellar MR images is the preferred image pre-processing technique for fractal analysis of the cerebellar white matter.

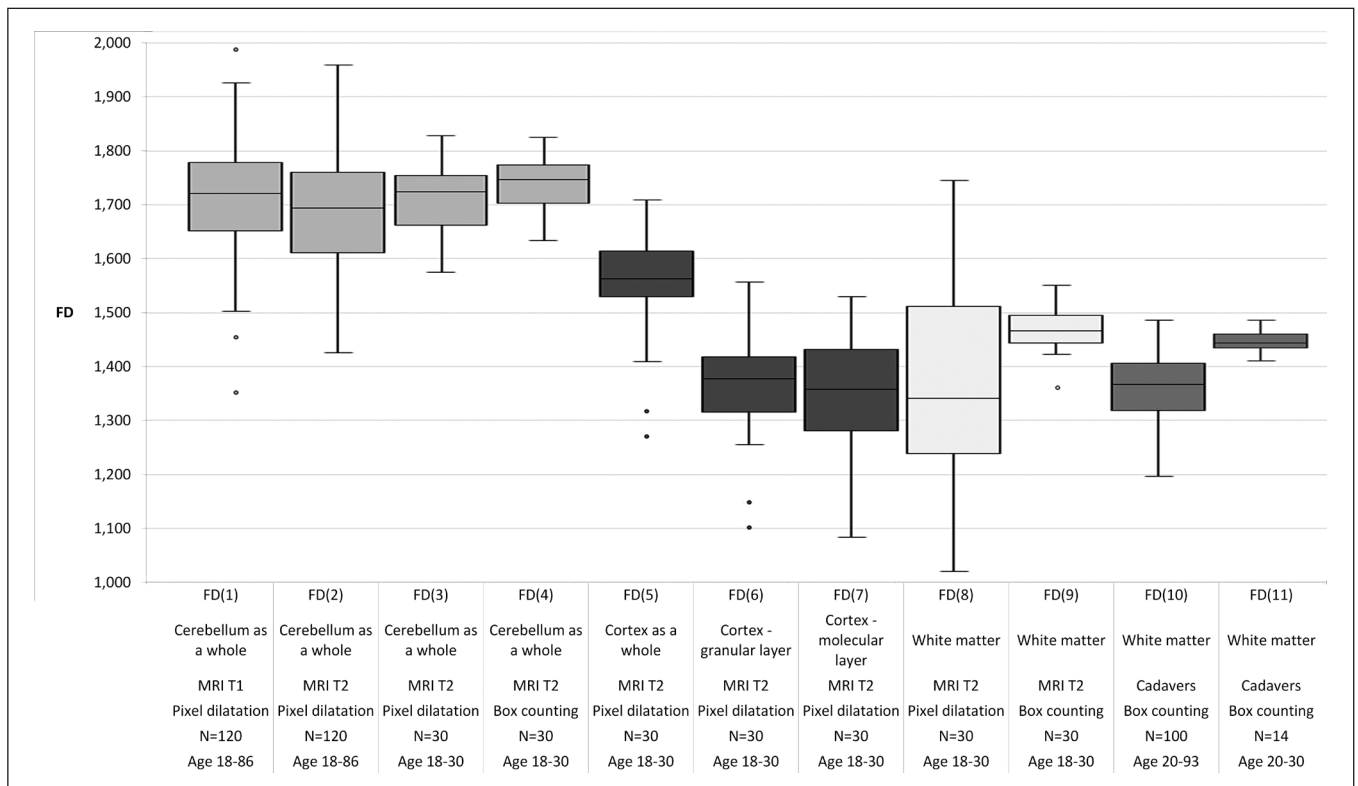


Fig. 3. The distribution of the fractal dimension values of human cerebellum.

Table I. The fractal dimension values of human cerebellum

FD	Cerebellar structure	Image type	Image preprocessing method	Pixels' luminance threshold	Fractal analysis method	Number of objects (N)	Age range, years	FD, M±m		
FD(1)	Cerebellar tissue as a whole	MRI T1	segmentation	100	pixel dilatation	120	18-86	1.714±0.009		
FD(2)		MRI T2						1.691±0.01		
FD(3)		MRI T2			segmentation	100	box counting	30	18-30	1.707±0.013
FD(4)										1.738±0.009
FD(5)	Cerebellar cortex as a whole	MRI T2	segmentation	100-80	pixel dilatation	30	18-30	1.564±0.018		
FD(6)	Cerebellar cortex – granular layer			90-80				1.377±0.02		
FD(7)	Cerebellar cortex – molecular layer			100-90				1.353±0.02		
FD(8)	Cerebellar white matter	MRI T2	segmentation	80	pixel dilatation	30	18-30	1.318±0.05		
FD(9)			skeletonizing					1.469±0.007		
FD(10)		Macro-photographs of cadaveric cerebella	visual assessment	box counting 14	100	20-95	1.372±0.006			
FD(11)					20-30	1.447±0.005				

DISCUSSION

Fractal analysis of MR brain images is an important area of modern neuroscience, since it allows diagnostics of various diseases of the nervous system. There are some studies which involved fractal analysis of human cerebellum [5-9]. The T1-weighted MR brain images were analyzed in all research works found in accessible literature. Different modifications of fractal analysis were applied: box counting [5-8] or pixel dilatation modification [9] and different fractal dimensions were determined: 2D (two-dimensional fractal dimension; the values vary from 1 to 2) [5, 6] or 3D (three-dimensional fractal dimension; the values vary from 2 to 3) [7-9]. Different components of cerebellar tissue were assessed (white matter and cortex), but FD of individual layers of cerebellar cortex were not measured in these studies.

In the studies of Akar E. et al. the 2D and 3D box counting methods were applied [5-7]. MR brain images were segmented into white matter, gray matter of cerebellum and cerebrospinal fluid. The mean value of 2D FD of cerebellar white matter was 1.49 ± 0.06 and the mean value of the 2D FD of cerebellar gray matter was 1.56 ± 0.05 [5, 6]. The mean value of 3D FD of cerebellar white matter was 2.26 ± 0.05 and the mean value of the 3D FD of cerebellar gray matter was 2.49 ± 0.04 [7].

In the study of Wu Y.T. et al. the 3D box counting method was utilized [8]. Automated 3D segmentation techniques were used; the cerebellar MR images were also segmented into white matter, gray matter and cerebrospinal fluid. The mean value of 3D FD of cerebellar white matter was 2.2746 ± 0.0446 and the mean value of the 3D FD of cerebellar gray matter was 2.5267 ± 0.0228 [8].

In the study of Liu J.Z. et al. the 3D pixel dilatation method was applied [9]. The image skeletonizing was used as a preprocessing method. The mean value of the 3D fractal dimension of the cerebellar white matter skeleton was 2.57 ± 0.01 [9].

Thus, the present study and the studies of other researchers demonstrate that the values of the fractal dimension of cerebellum and individual components of cerebellar tissue may be quite different depending on utilized modification of the fractal analysis (box counting or pixel dilatation, two or three dimensional fractal analysis), type of studied material (MRI or cadaveric specimens), MR sequence (T1 or T2), and the algorithms of image preprocessing (segmentation, skeletonizing, etc.).

CONCLUSIONS

1. The values of fractal dimension of cerebellar tissue as a whole determined on the T1- and T2-weighted MR brain images were not significantly different; both MRI sequences may be used to obtain the MR scans for the fractal analysis.
2. There was no significant difference between FD values measured on the same images but using different fractal analysis methods – pixel dilatation and box counting; both methods give comparable results.

3. Segmentation of the T2-weighted MR brain images using “threshold” tool according to pixel luminance is the preferable image preprocessing method for fractal analysis of cerebellar cortex as a whole, individual cortical layers and cerebellar tissue as a whole.
4. Skeletonizing of the MR images is the preferable image preprocessing method for fractal analysis of cerebellar white matter.
5. The algorithm of image preprocessing, MRI sequence and method of fractal analysis should be chosen according to the aim of study and features of the studied structure.

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ORCID and contributionship:

Nataliia I. Maryenko: 0000-0002-7980-7039^{A,D,F}

Oleksandr Yu. Stepanenko: 0000-0002-5686-0857^{A,C,F}

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CORRESPONDING AUTHOR

Nataliia I. Maryenko

Kharkiv National Medical University

4 Nauky Avenue, 61022 Kharkiv, Ukraine

tel: +380951405088

e-mail: maryenko.n@gmail.com

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ORIGINAL ARTICLE

DISORDERS OF ADAPTATION OF COMBATANTS AND THEIR MEDICAL AND PSYCHOLOGICAL REHABILITATION AT THE SANATORIUM STAGE OF TREATMENT

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Marianna V. Markova¹, Tetiana A. Aliieva¹, Artur R. Markov¹, Oleg A. Korop¹, Eugenia V. Lisovaya¹, Victoriia V. Babych², Svitlana O Vyazmitinova¹

¹KHARKIV MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KHARKIV, UKRAINE

²BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

The aim of the study was on the basis of the study of clinical manifestations and mechanisms of the formation of disorders of psychological adaptation among the combatants, to develop the principles and program of their medical and psychological rehabilitation.

Materials and methods: The study was attended by 153 people at the sanatorium and resort stage of treatment, of which 98 people were combatants and 55 people were civilians. The study used a set of methods aimed at the study of emotional disorders (HDRS, BDI, Spielberger Scale, "Asthenic state scale"), individual psychological features (Multilevel personal questionnaire "Adaptability," Test questionnaire G. Shmishek, K. Leonhard), features of psychosocial functioning (Mississippian scale of PTSD-military version) and quality of life (WHOQOL-BREF).

Results: At the stage of sanatorium treatment of combatants is important readaptation to peaceful living conditions. A clinical and psychological analysis of combatants showed that the presence of experienced state of subjective distress and emotional disorder leads to a decrease in the productivity of adaptation to a stressful event. The stress factor disrupted the integrity of the micro-social network of combatants and their system of social support and social values, with the prevailing sense of inability to overcome problems and build plans. A detailed analysis of the nature of depressive and anxiety manifestations was carried out, which allowed to determine the predictors of the violation of adaptation in combatants.

Thus, on the basis of the study of clinical manifestations and mechanisms of the formation of disorders of psychological adaptation among the participants of hostilities, the principles of their medical and psychological rehabilitation at the sanatorium and resort stage of treatment are developed.

Conclusions: The peculiarities of stress response in combatants as a component of personal potential play a role in the formation of predictors of psychological adaptation disorders among combatants. Psychological intervention at the tertiary stage of rehabilitation (sanatorium-resort) should be targeted, taking into account individual-psychological characteristics (preventors) and psychosocial factors (predictors)

KEY WORDS: stress reactions, disruption of psychological adaptation, post-traumatic stress disorder, stress, depression, anxiety, quality of life

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INTRODUCTION

Today, the population of Ukraine is massively under the influence of potentially traumatic events [1-6]. According to the UN data from April 14, 2014 to March 31, 2020, the total number of casualties from hostilities in the East of Ukraine was about 41-44 thousand people, of whom 13-13.2 thousand people were killed and 29-31 thousand people were wounded (UN, 14.04.2020) [7].

Psychological and psychopathological consequences of armed conflicts are manifested by a wide range of maladaptive reactions and painful conditions, the formation of which is determined by numerous factors [8-10]. At present, it is determined that post-war psychopathological changes in the form of disruption of psychological adaptation take place in the vast majority of combatants [11, 12].

The problem of preserving the health and efficiency of combatants participating in military conflicts is the main task of medical and psychological rehabilitation. However, the social and psychological aspects of the content and peculiarities of the implementation of complex programs of medical and psychological rehabilitation, focused on certain categories of wounded, on the specifics of manifestations of somatic and mental disorders among the combatants, are not developed enough today [13-19].

Today, rehabilitation and medical and psychological events for combatants are concentrated in the early stages, where numerous works are devoted to early response to mental disorders. Our attention was focused on the organization of medical and psychological rehabilitation of combatants at the sanatorium and resort stage, which is most close to a peaceful life.

THE AIM

The purpose of the study was to develop the principles and program of medical and psychological rehabilitation of combatants, based on the study of clinical manifestations and mechanisms of forming in them post-stress psychological disadaptation at the sanatorium-resort stage of treatment.

MATERIALS AND METHODS

The sample of the study was made up of 153 male persons, of whom 98 persons who were participants of hostilities in the area of the Joint Forces Operation in the East of Ukraine made up the main group, and the other 55 civilians who received spa treatment and had complaints about somatic and psycho-emotional state - a comparison group.

The following set of methods were used in the study: Hamilton's Scale of Anxiety and Depression (Hamilton, HDRS, HARS, 1967), A.T. Beck's Scale (BDI, 1961), C.D. Spielberger's Scale, Method "Asthenic state scale", Multilevel personal questionnaire "Adaptability", Mississippi PTSD scale (military variant), Test questionnaire G. Shmishek, K. Leongarda, WHOQOL-BREF. The results were processed using statistical methods using the computer program "SPSS for Windows"; The mathematical and statistical method was represented by a dispersive (used t-criterion of Studente, λ - criterion Kolmogorov-Smirnov, ϕ - Fischer) analysis.

The survey of combatants was carried out under the conditions of informed consent and with the implementation of international standards of bioethics. Diagnosis and basic treatment were conducted according to the criteria of diagnosis and treatment of psychiatric and behavioral disorders and clinical protocols for providing psychiatric care to the adult population of Ukraine.

The first stage of the study was aimed at screening complaints and forming groups according to the criteria for inclusion at the stage of sanatorium treatment in the SE "Clinical sanatorium" Resort Berezovsky mineral waters". The main group included 98 combatants, which are divided into two subgroups according to the indicators of stress response: with signs of disruption of adaptation - 74 people; with signs of PTSD - 24 people.

At the second stage, clinical manifestations and mechanisms of formation of disorders of psychological adaptation were studied.

The final stage of the study was the justification and development of the principles and program of medical and psychological rehabilitation among the participants of combat operations at the stage of sanatorium treatment.

RESULTS

Analysis of the nature of complaints among combatants at the stage of sanatorium treatment showed their diversity. On average, 89% had complaints in the form of: affective spectrum (depression, anxiety, fear, feeling confused, reduced mood background and irritability, flashes of ag-

gression) ideomotor-asthenic spectrum (excitement, rapid fatigue after mental activity and weakness after minimal physical effort, obsessive thoughts and memories, exhaustion of attention and memory) headaches, sleep disorders and frightening dreams and complaints of changes in the functioning of body systems. Whereas in the comparison group, somatic case complaints that had a chronic nature of the course of the disease were more prevalent.

Using the Mississippi scale, the PTSD military variant determined the degree of expressiveness of post-traumatic stress reactions in the main group of combatants at the stage of sanatorium treatment. For subgroups with signs of adaptation disorders, the symptoms of "avoidance" are more characteristic in 92% and in 85% of symptoms of physiological excitability, where lockdown in yourself, your feelings and thoughts lead to the formation of a sense of physiological tension and changes in perception and reactions. In subgroups with signs of PTSD, all symptoms are distinct, where the feeling of guilt in 87% and the symptoms of "invasion" in 100% are a common manifestation and moral regulator of socially acceptable behavior.

According to the objective assessment of the emotional state of the Hamilton scale, depressive symptoms prevailed in individuals of the main group: in 10% there were depressive disorders of moderate severity, in 71% - mild level. According to subjective assessment on the Beck scale, expressiveness varies between mild levels - in 43% and average manifestations of depression in 38%. Comparing the results with the results of a group of civilians, it was found that they had significantly lower depression indicators both by objective and subjective assessment: according to the Hamilton questionnaire, 62% had no depressive symptoms, and only 31% had mild depressive manifestations. According to the Beck scale, 58% - no manifestations of depressive symptoms and 36% - have mild subdepressant complaints associated with a somatic state.

The results obtained on the distribution of the level of depression in the subgroups of combatants indicate the severity of emotional experience and inhibition of the adaptation process. In the adaptation disorders subgroup by objective evaluation, 81% have a mild degree of depression, and according to subjective self-esteem, only 51% note the presence of mild levels of depression. In the subgroup with signs of PTSD, depressive symptoms prevailed by an average degree, both by an objective assessment of 37% and by subjective ones - by 50%. The mild level of depression was 62% on the Hamilton scale and 17% on the Beck scale, which generally reflects a more threatening state of the emotional sphere in the subgroup with signs of PTSD.

In 77% of participants of hostilities with signs of adaptation disorders, depressive symptoms are based on complaints of psycho-emotional tension, namely a feeling of tension, a decrease in performance, an unreasonable feeling of anxiety in the absence of a threat to the mental state of their health. Whereas for 82% of combatants with signs of PTSD, manifestations of depression are accompanied not only by physiological discomfort and changes in physical condition (inhibition, tension, lack of sleep, decreased

libido, general symptoms and somatic anxiety), but also by psychoemotional overload with guilt with suicidal thoughts.

An analysis of anxiety by objective evaluation in the main group showed that 36% of combatants had an average level of anxiety symptoms and 50.00% - an easy level, which was significantly more prevalent than in the comparison group, where 54% had mild anxiety manifestations. According to the Spielberger survey, 77% of the main group had high jet alarm rates and 63% - personal anxiety. In the group of comparison by objective assessment, alarming symptoms were obtained in 56%, and 45% - absent at all. Along with this, according to the questionnaire Spielberger, only 25% of civilians noted the average level of anxious jet-type symptomatic and 49% - personal anxiety.

The next stage of the study was to determine the level of quality of psychosocial functioning. The overall score of the quality of life of the respondents of the main group varied from 39 to 52 points. The average arithmetic estimate of quality of life was 43.6 points. In turn, the comparison group received high indicators of quality of life in 82%, where the average score was 79.3 points. Especially increased indicators of micro- and macro- social support. In the group of combatants with signs of adaptation disorders, the indicator of social well-being was especially reduced, which reflected the level of satisfaction with the environment, its position and capabilities. For combatants with signs of PTSD, the indicators of self-perception and microsocial support were reduced, due to increased criticism.

For combatants, the most inherent accentuations were demonstrative, alarming-stimulating type and stuck type. Whereas individuals of the comparison group are characterized by an exalted-emotive type of accentuation. In the subgroup of combatants with signs of adaptation disorders, the characteristic features of demonstrative, exciting, hyperthymic and cyclothymic types were characterized. Extreme expressiveness of these types of accentuations - expresses violations of affective-volitional processes that affect the control of behavior and characterize a certain behavioral style of realization of life position and personal needs. For combatants of PTSD, the types of accentuations of extreme degree of stuck, pedantic and exalted types that form the basis for the development of aggressive-fixative psychopathological symptoms aimed at themselves were inherent. Whereas in the comparison group the scale had an average range of expressiveness without extreme expressiveness, but the most characteristic scales are "sensitivity" ($12 \pm 0,58$ points) and "exalted" ($11,8 \pm 0,47$ points).

The study of personal adaptive potential of combatants showed a low level of regulation of behavior and communication orientation, as evidenced by low indicators on the "Adaptability" scale and "neuro-psychological instability." In the group of comparison, the whisky indicators on the scale of "behavioral regulation" and "adaptability" are obtained. In the subgroup with adaptation disorders signs, indicators of moral normality and behavioral regulation are significantly reduced, while in the subgroup with signs of PTSD, the indicator of communicative potential is reduced.

Analysis and generalization of the results made it possible to conclude that subgroups of combatants are characterized by different types of maladaptation disorders. For subgroup 1 there are psychotic reactions due to low indicators of neuro-mental instability, high conflictogenicity in interpersonal relationships and tendency to affective arousal; for subgroup 2 are asthenic reactions that indicate the type of maladaptive manifestations in the form of deterioration of somatic functioning, with a prevalence of deterioration in sleep, appetite, a sense of tension, a decrease in performance with hypochondriac fixation.

Next, we identified both predictors and preventors (protective personal resources) of post-stress psychological disadaptation in combatants at the sanatorium-resort stage of treatment.

For persons with signs of adaptation disorders in the system of formation of violation of adaptation, there are "distorted" thoughts about the displacement of the value of their role at the microsocial level, the loss of a certain significant position. Predictors of disruption of psychological adaptation among combatants with signs of adaptation disorders are:

- low indicators of moral normality that structure thoughts and help in interpreting and analyzing negative events of stressful level. Irrational perception of its role in the system of social functioning at micro and macro levels, which leads to a shift in the centralization of attention to aspects that will help restore the past system of life. On the one hand, this is a protective model of "avoiding" the problem, not the desire to face in thoughts with painful conclusions about his "I," which allowed to preserve the remnants of control of the situation and emotional comfort. And, on the other hand, it blocks the possibility of rethinking and transforming the experience gained, and in emotional regulation does not make it possible to verbalize their feelings and their origins.
- emotional instability, which is due to the personal radical of "exciting" and "cyclothymic-hyperthymic" types of accentuation, which leads to excessive affective excitation and subsequent exhaustion. Characterologically, the instability of emotional experience is determined, which does not allow to be fixed on a certain emotion and understand its formation, the causes of experience.
- psychotic type of reaction of maladaptation disorder, as a tendency to respond to high neuro-mental tension on the challenges of stressful situations, low tolerance to adverse factors and because of decreased motivation and productive activity.

The preventors of disruption of psychological adaptation are:

- sufficient level of communicative potential, which lies in the structure of personal adaptation potential. as the need for a social environment and the possibilities of implementation, that is, the possibility of receiving from the social environment a sense of support, reinforcing self-esteem, motivation for activity, the certainty of the goals of future projects.

- personal radical by "demonstrative" type of accentuation acts as a strong side, thereby achieving the result of significance at all social levels.

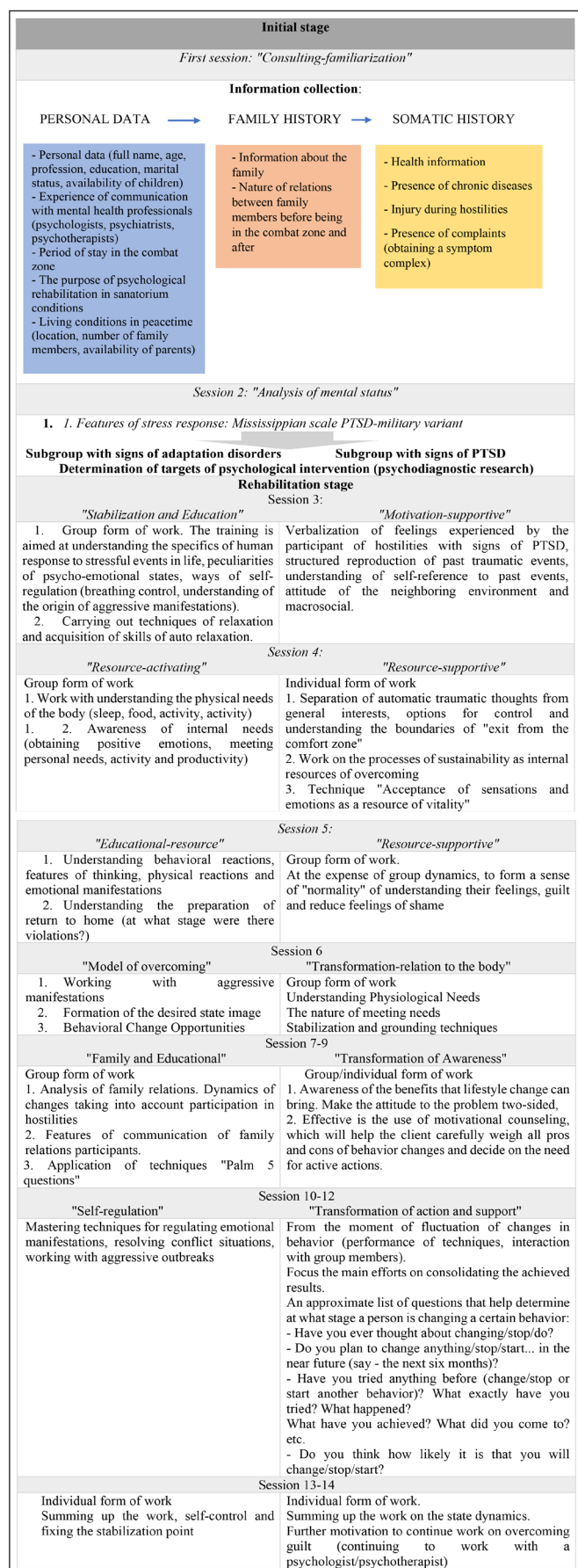


Fig. 1. Algorithm of medical and psychological rehabilitation of combatants at the stage of sanatorium treatment.

For a subgroup of combatants with signs of PTSD, "distorted automatic thoughts" there is a hyperbolized sense of guilt and shame. Predictors of disruption of psychological adaptation among combatants with signs of PTSD are:

low communicative potential, which, with a significant impact of stressogenic factors, isolates from close social interaction and prevents family members from affecting the emotional state. Support from the family is leveled and perceived as a trigger for irritation;

fixation of attention on the sharpness of emotional experiences, support in the tone of negative manifestations, as a form of punishment. It also acts as a way to maintain control of their values in the system of life and an attempt to reorient for the future. But the level of guilt is so high that it has no limit of punishment. Therefore, this method of emotional response very quickly leads to exhaustion at all levels, and mental and somatic;

asthenic reaction of maladaptive disorder, which immediately invades the system of satisfaction of basic needs and disrupts physiological functioning, shifting attention to somatic complaints.

The preventors of disruption of psychological adaptation among the participants of combat operations with signs of PTSD are established:

- moral normalcy, which ensures understanding of their position and adequately perceive the defined social role. In this case, the unfavorable role of the combatants is distinguished from their own internalized norms of accepted behavior. But it allows you to determine new norms;
- due to emotional fixation, the process of reevaluating past events, information received, understanding of their position takes place. That is, there is a constant search for a new understanding of the stressful situation that can satisfy the needs of the individual;
- an "exalted" type of accentuation, which acts as a certain social barrier, is directed to the desire for social usefulness, which corresponds to the vital position of combatants with signs of PTSD.

Based on the study of clinical manifestations and mechanisms for the formation of disorders of psychological adaptation, the principles of medical and psychological rehabilitation of combatants at the stage of sanatorium treatment, which included:

1. principle of maximum synergistic cooperation: doctor/psychologist - patient - therapeutic complex;
2. multi-level nature of sanogenic measures;
3. combined nature of biological and psychological-oriented influences;
4. the sequence and stage of the events;
5. an integrated approach to solving clinical problems, that is, a combined consistent effect on all etiopathogenetic links of functional disorders;
6. the principle of optimality of the use of medical-psychological, psychotherapeutic, natural, preformed, drug and non-drug factors;
7. the principle of structuring the construction of sessions (structured psychological interview).

Due to certain targets of psychocorrectional intervention, a program of medical and psychological rehabilitation of combatants was developed at the stage of sanatorium treatment, taking into account the presence of signs of RA and PTSD. This program includes three main stages: initial, rehabilitation and support. The structuring of the sessions of the medical and psychological rehabilitation program was in compliance with a certain algorithm of certain sessions, depending on the subgroup of the study with signs of PA or PTSD.

For persons with signs of RA, rehabilitation is aimed at stabilizing the psycho-emotional state through self-regulation skills, psycho-educational sessions aimed at understanding the peculiarities of conflict resolution, support of reflection with family members, understanding and assessing their condition with subsequent constructive response to symptoms.

For persons with signs of PTSD, rehabilitation was aimed at the resource-supporting aspect with the transformation of self-reliance and awareness of internal manifestations with the subsequent need for continued psycho-correctional intervention. Skills of self-regulation and grounding techniques were also acquired, in situations of high psycho-emotional stress.

The structuring of the sessions of the medical and psychological rehabilitation program was in compliance with a certain algorithm of certain sessions, depending on the subgroup of the study with signs of PA or PTSD (Fig. 1).

The effectiveness of these measures has been proven by improving the performance of reactive anxiety and quality of life, and reducing the feeling of asthenization. Participants of hostilities with signs of RA saw a decrease in the level of jet alarm in 50.0% (presence from 74.32% to 24.32%), no feeling of asthenization in 45.95% (a decrease from 58.11% to 12.16%) and an increase in the overall quality of life from 11.58 points to 17.85 points; in subgroup 2 with signs of PTSD decrease in reactive anxiety level in 50.0% (presence from 87.5% to 37.5%), there is no feeling of asthenization in 33.83% (decrease from 79.16% to 45.33%) and an increase in the overall quality of life from 10.37 points to 18.26 points.

DISCUSSION

We all face this problem when people who have participated in hostilities return to peaceful life, and it turns out that they need psychological help. In this case, it is confirmed that the cessation of participation in hostilities is not yet the cessation of the impact on the psyche of the same hostilities. All these consequences have participants in combat, in similar circumstances, and in other countries of the world. In the study on the results, we should note that the consequences have a prolonged effect. The results of the work of Svetlana Vyazmitinova proved that there are different options for violation of adaptation that require comprehensive assistance. It is very important that the system of sanatoriums is used, because this mechanism exists in our country and it is traditionally used to improve the state of health, because the influence of natural factors

has always been very significant for the human body. In this case, it is important to provide comprehensive assistance.

It is still noteworthy that, very often, as we see, the participants in the fighting do not recognize the need for psychological and psychiatric assistance. On this occasion, they avoid, to seek specialized assistance in psychiatric institutions. The use of the sanatorium allows you to naturally connect the mechanism of providing medical and psychological assistance to the complex of restorative institutions, without causing dissatisfaction with the fighters. The article thoroughly examines the components of personal, the basis of the occurrence of these psychological disorders. A phased model of assistance was worked out. This is very important, because it is a work for the future - the formation of new mechanisms, new adaptation skills that will help return the participants of the fighting to society, an effective peaceful life, because it suffers the most.

The period when our country got into a state of hostilities, we studied the experience of other countries that have faced this before. Undoubtedly, these are American studies, Israeli and Croatian. Our tactics regarding medical and psychological rehabilitation in relation to combatants were based on the studies of our colleagues. Now we have a rich personal experience. But the vast majority of works that would study the psychological state of combatants or active military, participating in hostilities, they relate to or the period of direct participation, or the period that arises after the end of participation in hostilities, that is, it is a military hospital, or just a return to peaceful life, if he is not somatically injured, and if he does not have clinically defined expressed disorders of the psychological sphere, then he comes home. But we understand that most disorders of psychological adaptation have a pre-natological level of impression. That is, such persons do not fall under the supervision of psychiatrists. And it is specialists in medical psychology that should work first. Another important point is that we have programs of medical and psychological rehabilitation, psychological correction of disorders of psychological adaptation of soldiers in the early stages of receiving specialized medical care. This work is the first to analyze the condition of the soldier at the stage of sanatorium treatment. New and important was found that most of the fighters, who already received specialized medical care, still got into sanatorium treatment with signs of disorders of psychological adaptation of different levels. The second value of Svetlana's work is that 2 clinical and psychological variants of disorders of psychological adaptation were determined, namely: with signs of adaptation disorders and with signs of PTSD, in this case it is not about clinically defined, but about pre-natological (syndromological) manifestations. This differentiation helped the author to determine the psychopathogenesis of these disorders, namely, to determine the predictors and preventors of each type of disorder and they turned out to be specific, differentiated for each of the options. This was the key to the development of a personalized program of medical and psychological rehabilitation. Now our medicine lives

in the trend of personification of medical care. Of course, each person is a separate world. While the nosological diagnosis may be the same, the approach to each patient should be as individual as possible. This work helps to personalize the approach to medical and psychological work with such patients.

CONCLUSIONS

The work analyzes the phenomenon of post-stress psychological disadaptation of combatants at the late - spa - stage of rehabilitation, and justifies the need to provide them with medical and psychological assistance at this stage, because the vast majority of modern research is devoted to the development of programs of medical and psychological rehabilitation, psychological correction of disorders of psychological adaptation of UBD in the previous, earlier stages of specialized medical care. Two clinical variants of the occurrence and course of pre-nosological manifestations of post-stress psychological disadaptation at the sanatorium stage of medical care - according to the type of disorder of adaptation with the predominance of the rod pathopsychological response such as "avoidance," and by the type of post-traumatic stress disorder with pathopsychological radicals "invasion" and "feelings of passage" were identified.

A program of medical and psychological rehabilitation of combatants at the stage of sanatorium treatment, taking into account the options of their stress response, which provides them with personalized medical care aimed at improving their rehabilitation potential, level of social functioning and quality of life, has been developed.

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ORCID and contributionship:

Marianna V. Markova: 0000-0003-0726-4925 ^{A,B,E}
 Tetiana A. Aliieva: 0000-0003-3511-027X ^{B,D,F}
 Artur R. Markov: 0000-0002-2164-7159 ^{B,D,F}
 Oleg A. Korop: 0000-0002-8833-4296 ^{E,F}
 Eugeniya V. Lisovaya: 0000-0003-4347-8453 ^{C,E,F}
 Victoriia V. Babych: 0000-0001-9899-0644 ^{B,D,F}
 Svitlana O Vyazmitinova: 0000-0001-6887-5477 ^{C,E,F}

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CORRESPONDING AUTHOR

Marianna V. Markova

Department of Psychotherapy of Kharkiv
Medical Academy of Postgraduate Education,
Street Amosova 58, 61176 Kharkiv, Ukraine
tel: +380506068145
e-mail: mariannochka1807@gmail.com

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ORIGINAL ARTICLE

DYNAMICS OF CHANGES OF C-REACTIVE PROTEIN LEVEL IN BLOOD SERUM IN THE DEVELOPMENT AND COURSE OF EXPERIMENTAL PERIODONTITIS AND THEIR CORRECTION BY FLAVONOL

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Andrii Demkovych¹, Petro Hasiuk¹, Yuliia Korobeinikova², Vitaliy Shcherba¹, Leonid Korobeinikov²¹I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE²POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

The aim: To study the value of C-reactive protein in the experimental animals blood serum with bacterial-immune periodontitis and its correction with quercetin.

Materials and methods: Modeling of periodontitis was performed by the following method: after thiopental anesthesia (at a dose of 40 mg / kg intramuscularly) rats were fixed. A subcostal injection of 0.01 ml of egg protein with cultures of *Streptococcus hemolytic* and *Staphylococcus aureus* at a dose of 4 CFU was performed in the area of periodontal tissues of the lower incisor as an initiating inflammatory factor. To enhance the immune process, a complete Freund's adjuvant was introduced into the animal's hind limb at the same time.

Results: Analysis of the results of the study of the content of C-reactive protein in the blood serum of animals with experimental bacteria and immune periodontitis, receiving injections of quercetin, showed a significant decrease by 1.31 times, compared with animals with this simulated pathology on the 14th day of the experiment without the use of flavonol. When comparing this indicator on the 14th day of development of experimental periodontitis with correction, it was found that it remained slightly higher than the indicators of the intact group of rats.

Conclusions: The level of C-reactive protein in the blood serum of experimental animals is an important indicator of the immune-inflammatory response, which increases its activation of the inflammatory system. The administration of flavonoid quercetin for 7 days helps to reduce the level of C-reactive protein in the blood serum of animals with experimental bacterial and immune periodontitis.

KEY WORDS: Protein level, Blood serum, Experimental periodontitis, Flavonol

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INTRODUCTION

Currently, there is an active search for new biomarkers of inflammation in patients with generalized periodontitis, with high prognostic value and assessment of factors, including carbohydrate metabolism disorders that affect the activation of various inflammatory mechanisms. Among the biomarkers known today, C-reactive protein (CRP) is probably one of the most promising indicators of inflammation and its definition occupies a special place among many clinical and laboratory tests. This is due to its ability to reflect the activity of the inflammatory process caused by bacterial infections and immunological diseases [1]. C-reactive protein is a short form of pentraxin that belongs to plasma proteins bound to calcium-dependent ligands, a superfamily of soluble pattern recognition molecules, and is mainly found as a pentameric compound [2]. The role of the protein is to facilitate the removal of microorganisms and necrotic tissues by activating cellular cytotoxic cascades. As a standard for assessing the level of systemic inflammation in patients, a highly sensitive C-reactive protein is used, which is not inferior in prognostic signif-

icance to other markers of inflammatory processes in the body. Microbe-inflammatory and destructive processes occurring in the body are characterized by changes in the concentration of plasma proteins [3].

In recent decades, the world medical community has paid special attention to the treatment and prevention of periodontal disease [4]. Eliminating the development and consequences of inflammatory processes in the periodontal complex means solving one of the global problems of oral health – tooth loss and, as a consequence, reduced masticatory efficiency and quality of life [5]. Therefore, an interesting and promising area is the study of organoprotective capabilities of quercetin, which has antioxidant, antitoxic, antiischemic, anti-inflammatory and membrane-stabilizing properties [6], which will determine new aspects of its use in patients of this category. The bioflavonoid quercetin belongs to the family of polyphenolic phytochemicals with pronounced antioxidant and anti-inflammatory properties. It is a powerful free absorber, antioxidant and anti-inflammatory agent [7]. The therapeutic effect of the drug, which belongs to the class of polyphenolic biofla-

Table I. Indices of C-reactive protein in the serum of experimental animals in different periods of development and course of experimental periodontitis and the use of quercetin ($M \pm m$)

Conditions and indicator of the experiment	Control group (intact animals)	White rats with experimental periodontitis			
		No correction		With correction	
Experiment duration (days)	-	7	14	30	14
Number of animals	10	8	8	8	8
C-reactive protein, mg / l	$0,45 \pm 0,01$	$0,75 \pm 0,02$ $p_1 < 0,01$	$0,67 \pm 0,01$ $p_1 < 0,01; p_2 < 0,01$	$0,64 \pm 0,02$ $p_1 < 0,01; p_2 < 0,01;$ $p_3 < 0,05$	$0,51 \pm 0,02$ $p_1 < 0,05; p_3 < 0,01$

Marking: p_1 - the significance of the differences with intact animals; p_2 - the significance of the differences with animals with experimental periodontitis on 7th day of the study; p_3 - the significance of the differences with animals with experimental periodontitis on 14th day of the study without correction by quercetin.

vonoids, is due to its anti-inflammatory and antioxidant activity. Quercetin reduces inflammation by inhibiting the production of cytokines such as interleukin-6, interleukin-8 and tumor necrosis factor, as well as by inhibiting the activation of nuclear factor κB (NF- κB) [8]. In addition, a study in an animal model of inflammatory pain origin showed that quercetin reduces pain, oxidative stress and cytokine production [9].

THE AIM

To study the value of C-reactive protein in the experimental animals blood serum with bacterial-immune periodontitis and its correction with quercetin.

MATERIALS AND METHODS

Experiments and research were conducted on the basis of the Central Research Laboratory (certificate of technical competence № 001/18 from 26.09.2018 to 28.12.2023) and the Interdepartmental training and research laboratory (certificate of technical competence № 132/17 from 29.12.2017 to 28.12.2022) of I. Horbachevsky Ternopil National Medical University on 42 nonlinear white mature male rats aged 7-8 months. Animals selected for the study were in the vivarium on a standard diet in accordance with sanitary and hygienic standards and GLP requirements. Operations were carried out in compliance with the general rules and provisions of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Animal Experiments (Kyiv, 2001).

Modeling of periodontitis was performed by the following method: after thiopental anesthesia (at a dose of 40 mg / kg intramuscularly) rats were fixed. A subcostal injection of 0.01 ml of egg protein with cultures of *Streptococcus hemolytic* and *Staphylococcus aureus* at a dose of 4 CFU was performed in the area of periodontal tissues of the lower incisor as an initiating inflammatory factor. To enhance the immune process, a complete Freund's adjuvant was introduced into the animal's hind limb at the same time.

These groups of animals were studied on the 7th and 14th days (groups II and III). Group IV animals were re-injected with adjuvant pathogen on the 14th day of the development of the inflammatory process in the periodontal complex and were examined on the 30th day. Due to this, an increase in the reproduction efficiency of bacterial and immune periodontitis was achieved. As established by our previous studies, microbial contamination coincided with that in humans [10].

The level of C-reactive protein in the serum was determined by enzyme-linked immunosorbent assay according to the instructions (High Sensitivity CRP (hs-CRP) Test System «Monobind Inc.», USA). The study was performed as follows: after selecting the required number of wells, added 25 mcl of standards, controls and test samples and 100 mcl of Enzyme Reagent CRP in each well. Carefully stir them for 20-30 seconds. Incubate for 15 minutes at room temperature. The contents of the wells were removed by aspiration. 350 mcl of wash buffer and 100 mcl of substrate working solution were added to each well, incubated for 15 min at room temperature. Stopped the development of color by adding to each well 50 mcl of stop solution and stirred for 15-20 seconds. The absorbance values of the wells at a wavelength of 450 nm were measured (measurements were performed at a reference wavelength of 620-630 nm). During the analysis, Streptavidine sorbed in the cells and biotinylated antibodies to the C-reactive protein interacted on the surface of the microwells. When mixing biotinylated antibodies and serum containing CRP antigen, between CRP antigen and antibodies there was a reaction to form an antibody-antigen complex. Sequentially, the biotin bound to the antibody interacted with Streptavidine deposited in the wells, resulting in immobilization of the complex. The results were displayed in mg / l [11].

Statistical processing of digital data was performed using the software «STATISTICA» 10.0 («Statsoft», USA) using variational-statistical methods of evaluation of the obtained data. The values of the arithmetic mean (M), its variance and error of the mean (m), the sample size (n) were calculated for all indicators. The reliability of the difference between the values of the independent quan-

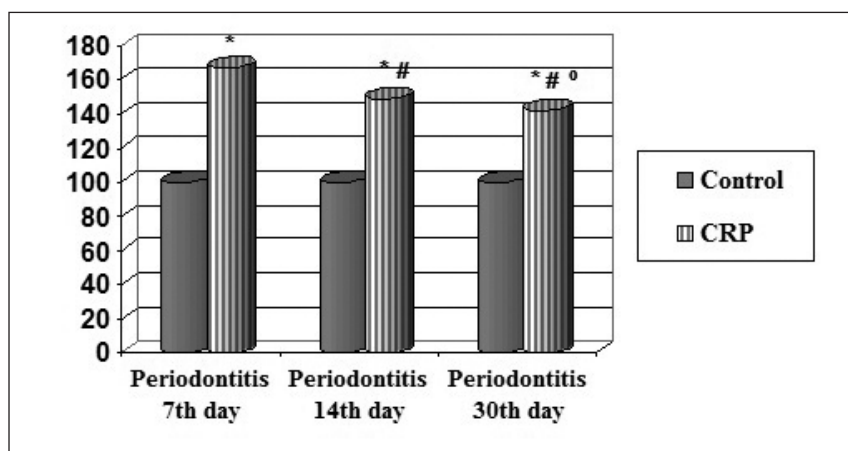


Fig. 1. Dynamics of C-reactive protein content in white rats blood serum with experimental periodontitis (% of control)

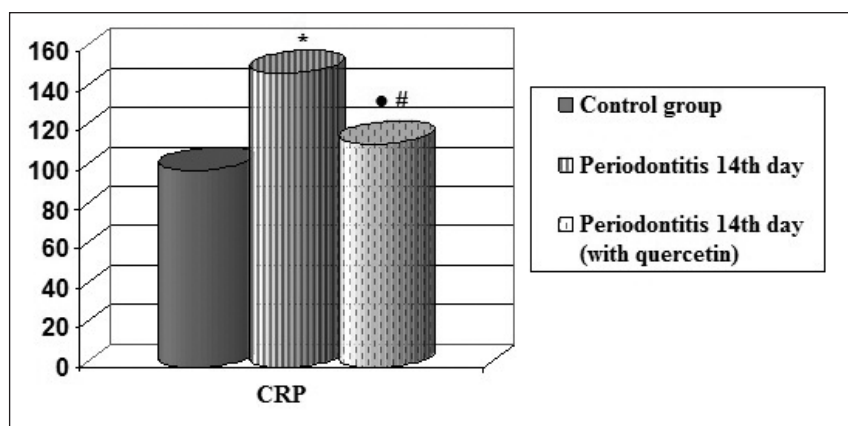


Fig. 2. The effect of quercetin on the content of C-reactive protein in white rats blood serum with experimental periodontitis (% of control)

titative values was determined at the normal distribution by the Mann-Whitney U-test. The difference in the results of the study was considered probable when the reliability coefficient was less than 0.05 [12].

RESULTS

Regarding the change in the content of C-reactive protein in the serum of experimental animals with periodontitis, it should be noted that its content on the 7th day of the experiment significantly exceeded (by 1.67 times; $p < 0.01$) indicators that were in animals of the control group (Table I, Fig. 1).

In the rats of the experimental group, which were observed on the 14th day of the study, compared with the group of animals that were studied on the 7th day, there was a statistically significant increase the level of C-reactive protein in blood serum (by 1.12 times; $p < 0.01$) (Fig. 1).

Also in this period there was a probable increase in the values of this indicator of inflammation (by 1.49 times; $p < 0.01$) relative to the corresponding control. Analysis of the results obtained on the 30th day of the study showed a similar nature of changes, ie a probable increase in C-reactive protein, relative to the data of the control group (by 1.42 times; $p < 0.01$).

The determination of the level of C-reactive protein in the serum on the 30th day of the experiment showed that its content in the blood was significantly lower than the

data on the 7th and 14th day of observation – by 1.17 times ($p < 0.01$) and by 1.05 times ($p < 0.05$), respectively.

Analysis of the results of the study of the content of C-reactive protein in the blood serum of animals with experimental bacteria and immune periodontitis, receiving injections of quercetin, showed a significant decrease by 1.31 times ($p < 0.01$), compared with animals with this simulated pathology on the 14th day of the experiment without the use of flavonol (Table I).

However, when comparing this indicator on the 14th day of development of experimental periodontitis with correction, it was found that it remained slightly higher (by 1.13 times; $p < 0.05$) than the indicators of the intact group of rats (Fig. 2).

DISCUSSION

One of the indicators of tissue damage in inflammatory processes is C-reactive protein, which belongs to the so-called acute phase proteins. C-reactive protein is involved in the interaction of T- and B-lymphocytes, activates complement in the classical way. C-reactive protein stimulates protective reactions, activates immunity [13]. CRP is produced in the liver and in much smaller numbers by peripheral blood lymphocytes, during acute episodes of inflammation or infection. Determination of serum C-reactive protein level is used in the clinic as a non-specific marker for inflammation, infection and tissue damage

associated with the acute phase [14]. It should be noted that CRP is a biomarker of elevated levels of IL-1, IL-6 and TNF- α . In addition to its role in humoral innate immune responses, C-protein recognizes and binds several intrinsic ligands, such as the complement system, leading to a significant increase in infarct size, cell receptors, apoptotic cells, growth factors, and extracellular matrix components, and therefore contributes to the progression of cardiovascular disease [15]. Inflammation releases interleukin-6 and other cytokines that cause CRP and fibrinogen synthesis in the liver. In the process of inflammation, C-reactive protein stimulates the synthesis of pro-inflammatory factors: the release IL-1 β , IL-6, TNF- α by monocytes, the expression of human endothelial cells of adhesion molecules and protein chemotaxis of monocytes-1 [16]. It also activates cholesterol synthesis, reduces the expression and activity of nitrogen monoxide (NO) in the vascular endothelium. Thus, CRP is not only a marker of the inflammatory process, but also its inducer. C-reactive protein plays a key role at all stages of this inflammatory process: it stimulates immune responses, including phagocytosis, participates in the interaction of T- and B-lymphocytes and can actively influence the activation of the complement system by inducing apoptosis, vascular cell activation, involvement leukocytes, lipid accumulation, platelet aggregation [17]. On the surface of many bacteria, the C-reactive protein forms compounds with phosphatidylcholine molecules that are strong opsonins, ie antibodies and complement factors that enhance macrophage phagocytosis and stimulate the digestion of microorganisms. C-reactive protein is a very sensitive element of blood, one of the first to respond to tissue damage [18, 19]. The presence or increase in the level of CRP in the serum is a sign of inflammation, damage, penetration of foreign microorganisms, parasites and fungi. Thus, increasing the concentration of CRP is a biochemical marker of the development of both the inflammatory process in the body in general and periodontitis in particular.

CONCLUSIONS

1. The level of C-reactive protein in the blood serum of experimental animals is an important indicator of the immune-inflammatory response, which increases its activation of the inflammatory system. The development and course of simulated inflammation of bacterial-immune genesis is accompanied by an increase in serum concentrations of CRP throughout the period of formation of the inflammatory focus in the periodontal complex, which is associated with the response of innate humoral immune responses to antigen stimulation.
2. The administration of flavonoid quercetin for 7 days helps to reduce the level of C-reactive protein in the blood serum of animals with experimental bacterial and immune periodontitis, which may be a sign of stabilization and attenuation of the inflammatory process and one of the indicators of its effective influence on this pathogenetic link of inflammatory process in the periodontal complex.

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ORCID and contributionship:

Andrii Demkovych: 0000-0001-9823-4283 ^{A,F}

Petro Hasiuk: 0000-0002-2915-0526 ^D

Yuliia Korobeinikova: 0000-0001-9247-1448 ^B

Vitaliy Shcherba: 0000-0002-1998-5183 ^E

Leonid Korobeinikov: 0000-0002-4964-2122 ^C

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CORRESPONDING AUTHOR

Petro Hasiuk

I. Horbachevsky Ternopil National Medical University

1 Maidan Voli, 46001 Ternopil, Ukraine

tel: +380961445444

e-mail: gasiukpa@tdmu.edu.ua

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ORIGINAL ARTICLE

PATHOMORPHOLOGICAL FEATURES OF GASTROESOPHAGEAL REFLUX DISEASE REALIZATION IN YOUNG PEOPLE WITH AUTOIMMUNE THYROIDITIS

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Tamara M. Pasiieshvili, Tetiana V. Bocharova, Natalia M. Zhelezniakova, Lyudmila M. Pasiyeshvili
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

The aim: To evaluate the pathomorphological features of the esophageal mucous membrane in young people with GERD and autoimmune thyroiditis.

Materials and methods: 120 patients with GERD and AIT and 45 people with isolated GERD matched for age, gender and social status were examined. Esophagogastroduodenoscopy, histological study and comparative morphometry of the esophageal mucosa were performed.

Results: The frequency of erosive GERD in the examined groups of patients did not statistically differ. At the same time, integral analysis of the structure of erosive forms of GERD revealed statistically significant redistribution of grades of esophagitis towards its enhancement in patients with comorbid pathology. The histological study showed that in patients with GERD and AIT all the morphometric parameters studied had a significantly more severe course and exceeded similar indicators of the group with isolated GERD: epithelium total thickness, epithelium basal layer thickness, connective tissue papillae height, intercellular space. The analysis of morphological changes frequency showed that epithelium basal layer hyperplasia, dystrophic changes and epithelial edema, elongation of papillae and dilation of intercellular space were significantly more frequent in the group with comorbid pathology.

Conclusions: GERD and euthyroid AIT comorbidity in the student population is accompanied by a statistically significant redistribution of esophagitis grades towards its aggravation. The presence of concomitant euthyroid AIT in patients with non-erosive GERD leads to statistically more pronounced disorganization of esophageal mucosal epithelium.

KEY WORDS: gastroesophageal reflux disease, autoimmune thyroiditis, oesophageal biopsy, pathomorphological study, young population

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INTRODUCTION

The traditional interpretation of gastroesophageal reflux disease (GERD) etiopathogenesis as a chronic disease caused by disorders of gastroesophageal motor-evacuation function with regular reflux of gastric and/or duodenal contents into the esophagus has undergone significant changes [1-3]. Not rejecting the importance of chemo-mechanistic aspects («chemical burn» theory) in the development of GERD, modern studies supplement the pathogenesis of the disease with new data on the role of the chronic inflammatory process in the course of nosology; there is an assessment of universal mediators and the search for esophageal-specific mediators of inflammation and sources of their production.

According to the classical «chemical burn» theory, acid-induced death of superficial cells of the esophageal epithelium was thought to provoke an acute granulocytic inflammatory response, which begins in the epithelium and then progresses to the mucosal lamina, with the formation of a defect in the submucosal layer. It has also been suggested that loss of superficial cells of the esophageal mucosa stimulates hyperplasia of progenitor cells in the basal layer of the squamous epithelium, which is a characteristic histological sign of GERD [4-6].

In 2009, the pathogenetic concept of the «chemical burn» was challenged in an animal model of GERD caused by esophagoduodenostomy [7]. Reflux esophagitis in rats did not start from superficial cell death and epithelial infiltration by granulocytes, but rather from T-lymphocytes that first infiltrated the esophageal submucosa and then infiltrated the lamina propria and epithelium. Superficial erosions did not appear until several weeks after esophagoduodenostomy, and basal cell hyperplasia occurred long before the loss of mucosal superficial cells. A culture study of human esophageal epithelial cells found that acids and bile salts caused the release of pro-inflammatory and proliferative cytokines, such as interleukin-8. Based on such data, an alternative hypothesis of GERD pathogenesis was proposed, in which refluxate did not directly destroy esophageal epithelial cells, but rather stimulated them to secrete cytokines [8-10]. The latter caused proliferative changes in the epithelium and mobilized T-lymphocytes and other inflammatory cells, which eventually damaged the mucosa [11-13].

An important factor in pathomorphological realization of GERD may be its combination with other diseases, including autoimmune thyroiditis (AIT), which creates unfavorable immune and humoral background that may contribute to the worsening of GERD course [14,15].

Table I. The incidence structure of different esophagitis grades in the examined patients

Esophagitis grades	GERD and AIT (n=34)	GERD (n=11)	Significance of differences 1
A	6 (17.7%)	7 (63.6%)	df=3 $\chi^2=8.772$ p=0.033
B	18 (52.9%)	3 (27.3%)	
C	8 (23.5%)	1 (9.1%)	
D	2 (5.9%)	0 (0%)	

Note: p<0.05 – the difference is statistically significant between groups

Table II. Mucous membrane morphometric parameters of the distal part of the esophagus in the studied patients, M±m

Groups	GERD (n=35)	GERD+AIT (n=50)	Significance of differences 1
Epithelium total thickness, μm	286.1±8.2	319.3±9.1	p<0.01
Epithelium basal layer thickness	μm 49.7±2.1	79.6±3.2	p<0.01
	% 17.3±0.3	25.1±2.9	p<0.01
Connective tissue papillae height	μm 172.7±4.6	224.8±7.3	p<0.01
	% 60.4±3.3	72.3±3.1	p<0.01
Intercellular space, μm	1.12±0.09	1.55±0.11	p<0.01

Note: p<0.05 – the difference is statistically significant between groups

THE AIM

The objective of the study was to evaluate the pathomorphological features of the esophageal mucous membrane in young people with GERD and autoimmune thyroiditis.

MATERIALS AND METHODS

The study was conducted at the Department of General Practice - Family Medicine and Internal Diseases, the Department of Pathological Anatomy, the Department of Internal Medicine no. 1 of Kharkiv National Medical University, Ukraine, between 2017 and 2019. The study was approved by the Ethics and Bioethics Committee of Kharkiv National Medical University. All the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

Criteria for inclusion: students 18 - 25 years old; verified diagnosis of GERD; for the main group - verified diagnosis of AIT.

Criteria for exclusion: hypothyroidism, hyperthyroidism, other endocrine and GIT pathology; diseases of the cardiovascular system, kidneys, lungs; cancer; mental illness; pregnancy and lactation; minority; patient's refusal to participate in the study.

165 patients were examined, including 120 patients with comorbidity of GERD and AIT (main group) and 45 people with isolated GERD (comparison group). The mean age in the groups was 21.9 ± 2.7 and 21.2 ± 2.4 years, respectively (p>0.05). The contingent was represented by students from various universities of Kharkiv (Ukraine); 93 patients (77.5%) of the main group and 34 examined persons (75.56%) of the comparison group were women, 27 (22.5%) and 11 (24.44%) respectively were men. Standard

values were obtained while examining 20 almost healthy patients of the same age, gender and social status.

Autoimmune thyroiditis was confirmed by the presence of antibodies to thyroperoxidase and thyroglobulin and thyroid gland ultrasound. The functional state of the thyroid gland was assessed in the previous stages of the study on the content of thyroid-stimulating hormone, free triiodothyronine and thyroxine, all patients were diagnosed with euthyroid status.

The diagnosis of GERD was confirmed by typical complaints, history, clinical and instrumental data. Visual assessment of the esophageal mucous membrane was performed by endoscopic examination of the esophagus (videoendoscopic system "Fuginon", Japan) with biopsy and subsequent histological examination of the material.

Material for the histological study was taken from the mucous membrane of the distal esophagus 3 cm above the conventional circular line connecting the stomach and esophagus. Pieces of the mucosa of the distal esophagus were fixed in formalin, passed through alcohols in increasing concentration, embedded in paraffin and prepared 5 μm thick sections that were stained with hematoxylin-eosin and picro-fuchsin according to Van Gieson method. Microscopic examinations were performed on an Olympus BX-41 microscope. Morphometric parameters were obtained using the Olympus DP-Soft (Version 3: 1). The total thickness of the epithelium, basal layer thickness, the height of connective tissue papillae and intercellular space were determined in 10 random fields of view in high (x40 lens, x10 eyepiece).

Statistical processing was performed using Statistica software. The results were analyzed using methods of descriptive statistics: calculation of arithmetic mean, 95% confidence interval, and standard error in the sample. Differences obtained by paired comparisons were considered statistically significant at p < 0.05.

Table III. The frequency of morphological changes of the esophageal mucosa in the studied patients

Sign	GERD (n=35)		GERD and AIT (n=50)		Significance of differences (χ^2) ¹
	Abs.	%	Abs.	%	
Epithelium basal layer hyperplasia	22	62.9	44	88.0	df=1 $\chi^2=7.499$ p=0.006
Epithelial edema	21	60.0	41	82.0	df=1 $\chi^2=5.049$ p=0.025
Connective tissue papillae elongation	20	57.1	41	82.0	df=1 $\chi^2=6.278$ p=0.012
Submucosal fibrosis	19	54.3	36	72.0	df=1 $\chi^2=2.829$ p=0.093
Intercellular space dilation	19	54.3	39	78.0	df=1 $\chi^2=5.342$ p=0.021
Dystrophic changes	23	65.7	45	90.0	df=1 $\chi^2=7.589$ p=0.006
Leukocyte infiltration	22	62.9	39	78.0	df=1 $\chi^2=2.33$ p=0.127

Note: $p < 0.05$ – the difference is statistically significant between groups

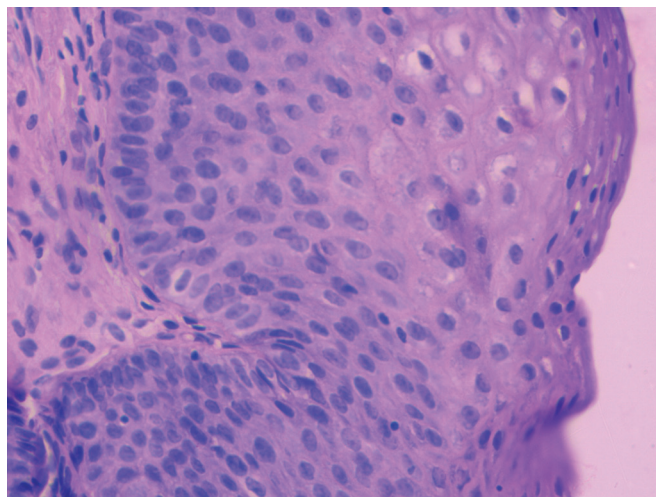


Fig. 1. Esophageal epithelium thickening due to the basal zone hyperplasia in a patient with comorbid pathology (staining with hematoxylin and eosin, x 400)

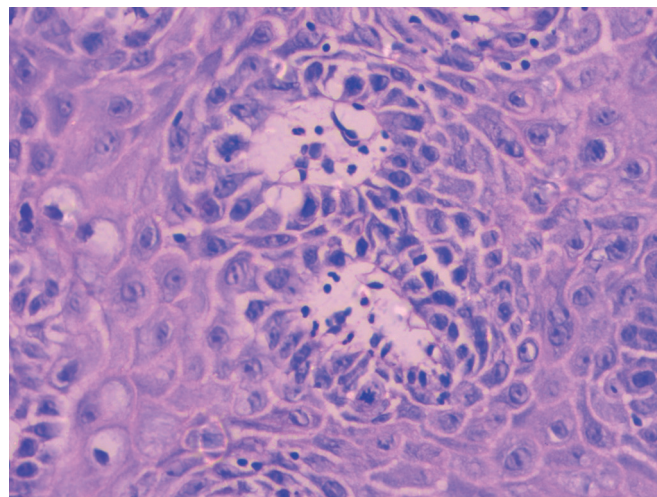


Fig. 2. Leukocyte infiltration of the esophageal mucosa with the presence of rare eosinophils in a patient with comorbid pathology (staining with hematoxylin and eosin, x 400)

RESULTS

The endoscopic examination of patients with comorbid GERD and AIT revealed erosive lesions of the esophagus in 34 (28.3%) and nonerosive – in 86 (71.7%) cases. In the comparison group, erosive GERD was found in 11 (24.4%) and nonerosive – in 34 (75.6%) patients. The grade of esophagitis was determined according to the Los Angeles classification (Tab. I).

The frequency of erosive GERD in the examined groups of patients did not statistically differ ($df=1$, $\chi^2=0.250$,

$p=0.618$). At the same time, integral analysis of the structure of erosive forms of GERD revealed statistically significant redistribution of grades of esophagitis towards its enhancement in patients with comorbid pathology ($df=3$, $\chi^2=8.772$, $p=0.033$).

Taking into account the fact that erosive esophageal lesions were observed in a small number of patients, who also had different grades of esophagitis, which made statistical processing impossible. Thus, samples of patients

with a non-erosive form were chosen for histological study: 35 cases of isolated GERD and 50 – of GERD and AIT comorbidity.

The following histological peculiarities of esophageal mucosal epithelium were noticeable during the examination of biopsy material in both groups of patients. First of all, significant changes in multilayer squamous epithelium were noted: quite often stratification of its layers was disturbed, basal zone hyperplasia and elongation of stromal papillae were noted.

As a rule, the thickness of the basal layer is formed by a few cells and is less than 15% of the total thickness of the epithelium, and the length of the papillae does not exceed 50%¹⁶⁻¹⁸. In the studied groups, the thickness of the basal epithelium zone was greater both in absolute value and in relation to the total thickness of the epithelium. (Tab. II)

It should be noted that in GERD and AIT group, the epithelium thickening was mainly due to the basal layer, which was 25.1%, whereas in GERD isolated group this index was close to the physiological norm at the level of 17.3% (Fig.1).

During the histological examination, it was noticed an increase in the size of the nuclei, their hyperchromicity, the presence of physiological mitoses, which also indicated the activation of regenerative processes. Characterizing histological changes in epithelial cells in patients with isolated GERD, we should note significant dystrophic and focal necrotic changes on the background of pronounced edema of the spinous and basal layers.

At the same time, in patients with GERD and AIT comorbidity, epithelial cells were polymorphic with cytoplasm vacuolization, some of them were sharply increased in size with signs of parenchymatous protein (hydropic) degeneration and nuclear dislocation to the cell periphery. The above changes in the epithelium were observed in combination with significant intercellular edema with loss of normal orientation of superficial epithelial cells, in some places with the presence of intercellular bridges.

Morphologically, a significant increase in the length of papillae was detected both in isolated GERD and in combined pathology. However, an intergroup comparison revealed that in patients with GERD and AIT the thickness of the basal epithelial layer and the length of connective tissue papillae was significantly greater (Tab.II).

It should be noted that the increased thickness of the basal layer may reflect the increased proliferative activity (intensification of regenerative processes) of its cells. The length of connective tissue papillae reached 75% of the epithelial layer, and its increase is most likely explained by secretion of inflammatory mediators, stimulating proliferation of fibroblasts, endothelium and smooth muscle cells. The submucosal layer showed morphological signs of fibrosis with sclerotic changes in the lamina propria with thickening of collagen fibers, which also can cause changes in the shape and length of villi.

The leukocytic infiltration in submucosa was less pronounced in the group with isolated GERD, where lymphocytes and macrophages predominated in the infiltrate. In the group of combined pathology, infiltration was more intensive and polymorphic, the

presence of neutrophilic leukocytes, especially in foci of dystrophic and necrotic changes was determined, accumulation of eosinophils was observed in some preparations (Fig. 2).

Thus, the main morphological signs of reflux esophagitis in both groups were: basal layer hyperplasia; elongation of connective tissue papillae; intercellular edema with the intercellular space dilation; dystrophic changes with cytoplasm vacuolization, and in some places focal necrotic changes of epithelial cells; the presence of marked inflammatory infiltration in submucosa layer. In spite of the fact that above mentioned signs were noticed both in patients with a combination of GERD and AIT and with isolated GERD, the intergroup comparison revealed some significant differences in these values (Tab. III).

Thus, epithelium basal layer hyperplasia, dystrophic changes and epithelial edema, elongation of papillae and dilation of intercellular space were significantly more frequent in the group with comorbid pathology. Besides, patients with GERD and AIT had a higher frequency of inflammatory leukocytic infiltration (78%) combined with signs of submucosal fibrosis (72%) in comparison with the group of isolated GERD – 62.9% vs 54.3% respectively, however, these differences were not significant.

DISCUSSION

Research data of recent years indicate significant progress in the study of pathogenetic links, pathways and mechanisms of GERD progression, naturally accompanied by optimization of diagnostic approaches and therapeutic strategy. Therefore, in recent years, the increasing attention of scientists is attracted by the comorbid course of GERD with other diseases. This problem is of particular importance when combining with autoimmune pathology, which is usually associated with the development of systemic inflammation that can act as an additional factor of GERD progression. The development of GERD in young people is of great importance, since the formation of chronic pathology at an early age, especially in the presence of such “insidious” companion as AIT, may be accompanied by rapid progression of nosology and early development of complications.

However, at the current stage of medical science development, there are practically no studies of pathomorphological features of the esophagus in young patients with GERD and AIT comorbidity.

The presented study identified the significant dilation of the intercellular space in patients of both groups, which is considered by the overwhelming majority of scientists to be a classic marker of GERD. At the same time, some authors believe that dilation of the intercellular space in the esophageal epithelium is not a pathognomonic sign of GERD and can be observed in patients with psychological stress [19], which is relevant to the student population and may have been an additional trigger in this category of patients. In addition, according to Lori A Orlando et al., dilation of the intercellular space may be a sign of epithelial barrier disorder due to increased intercellular permeability [20].

The study of Jeremy R Parfitt et al. compared the histological picture of esophageal mucosa in GERD and in eosinophilic esophagitis and showed the commonality of certain changes for both nosologies – elongation of villi, hyperplasia of the mucosal basal layer and intercellular edema [21]. Meanwhile, the key differential diagnostic criteria for eosinophilic esophagitis are the presence of 15 or more eosinophils in the field of view, eosinophilic micro abscesses, superficial eosinophilic infiltrates and eosinophil degranulation. Eosinophilic esophagitis is known to be an immune-mediated inflammatory disease of the esophagus, so the presence of eosinophil accumulation in selected preparations of patients with comorbid pathology may have been a consequence of the influence of an additional autoimmune inflammatory component brought on by AIT.

It should be noted that eosinophils are physiologically present in the GI tract, but their presence in the esophagus is pathological. Eosinophilic infiltration leads to thickening of the esophageal mucosa, basal layer hyperplasia, and villous deformity [22]. In addition, eosinophils are able to secrete cytotoxic granules, directly act on neurons and damage axons of the esophageal sphincter muscle fibers. The latter leads to decreased tone and progression of GERD [23,24]. The presence of chronic inflammation in the mucosa and submucosa layer of the esophagus also leads to the activation of fibroblasts and causes the formation of fibrosis and esophageal stenosis [25].

CONCLUSIONS

GERD and euthyroid AIT comorbidity in the student population is not associated with the prevalence of an erosive form of GERD, but it is accompanied by a statistically significant redistribution of esophagitis grades towards its aggravation.

The presence of concomitant euthyroid AIT in patients with non-erosive GERD leads to statistically more pronounced disorganization of esophageal mucosal epithelium due to basal layer hyperplasia, edema of spinous and basal layers, dilation of intercellular space, elongation and deformity of connective tissue papillae.

Signs of submucosal fibrosis, marked dystrophic and in some places, necrotic changes in epithelial cells and inflammatory infiltration in the submucosal layer should also be considered as characteristic features of GERD in the examined patients, meanwhile, the presence of concomitant euthyroid AIT non significantly, but increases the expressiveness of these deviations.

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ORCID and contributionship:

Tamara M. Pasiieshvili: 0000-0002-7079-4761 ^{A-F}

Tetiana V. Bocharova: 0000-0002-2264-1744 ^{A-B,D-E}

Natalia M. Zhelezniakova: 0000-0002-5786-9378 ^{A,B,D-F}

Lyudmila M. Pasiyeshvili: 0000-0001-7527-782X ^{A,D-F}

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Tamara M. Pasiieshvili

Kharkiv National Medical University
4 Nauki Ave., 61022 Kharkiv, Ukraine
tel: +380505950303
e-mail: pasotoma2017@gmail.com

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ORIGINAL ARTICLE

PECULIARITIES OF DISTRIBUTION OF ANTIBIOTIC RESISTANT STRAINS OF *E. COLI* - *E. FAECALIS* ASSOCIATION IN THE UROGENITAL TRACT OF PREGNANT WOMEN

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Olha S. Voronkova, Maksym V. Lusta, Yuliia S. Voronkova, Yelyzaveta S. Fawzy, Tetiana H. Ostanina

OLES HONCHAR DNIPRO NATIONAL UNIVERSITY, DNIPRO, UKRAINE

ABSTRACT**The aim:** To investigate the prevalence of antibiotic-resistant strains from the association of *E. coli* - *E. faecalis* in the urogenital tract of pregnant women.**Materials and methods:** Used bacteriological method of sectoral culture of urine on nutrient media and identification of strains to the species. The susceptibility of strains to antibiotics was determined by disk-diffusion method, the interpretation of results - according to current EUCAST recommendations. Statistical processing was performed in MS Excel (Analysis of variance (ANOVA)).**Results:** The number of resistant to cefuroxime, cephalexin, nitrofurantoin, norfloxacin and trimethoprim uropathogenic strains of *Escherichia coli* does not change depending on the monostrain and associated strain of *E. coli* from the urine of pregnant women in Dnipro. It was found that the differences in the number of fosfomycin-resistant uropathogenic strains of *E. coli* depending on the type of culture - the number of fosfomycintolerant urostrains *E. coli* is 2 times higher among the associated strains, compared with monostains of the studied microorganism. The number of uropathogenic strains of *E. faecalis* resistant to ampicillin, trimethoprim, nitrofurantoin and norfloxacin does not change depending on the type of culture isolated from the urine of pregnant women in Dnipro.**Conclusions:** The symbiotic type of existence of two representatives of the normal intestinal flora on the uroepithelium of pregnant women may be associated with the development of *Escherichia coli* antibiotic resistance only to fosfomycin compared with mono-infection caused by each of these microorganisms.**KEY WORDS:** antibiotic resistance, pregnancy, urogenital tract, urine, fosfomycin-resistants

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INTRODUCTION

Uncontrolled use of ABD in everyday life, the lack of a well-established prescription release in pharmacies, leads to the creation of a reservoir of resistance determinants among pathogenic and, above all, opportunistic bacteria [1-3]. Bacterial resistance to antibiotics is considered one of the main modern problems of clinical microbiology and general therapeutic activity of clinicians. The development of bioengineering and modern biotechnological progress contribute to the discovery and development of new antibacterial drugs (ABD) in the pharmaceutical market in Ukraine and the world, but not all antibiotics are widely used clinically [4, 5]. The problem with antibiotic resistance is primarily that bacteria have a high capacity for phenotypic and genetic variability. They are able to adapt even to those ABD that are considered universal and starting in the treatment of bacterial infections [6, 7]. Bacteriologists and epidemiologists are increasingly registering strains of *Escherichia coli* and *Enterococcus faecalis* that become resistant to antibiotics. These microorganisms are opportunistic pathogens for humans and are in close symbiosis with our body, playing a role in the digestive system and the creation of colonization resistance of the gastrointestinal mucosa [2, 8, 9]. Usually these microorganisms are considered to be representatives of the normal intestinal flora, however,

some strains of these bacteria are already polyresistant. This has no effect on the breakdown function of nutrients, but when immunoreactivity is impaired, they can alter loci of residence, causing infections such as urinary tract infections (UTI) in pregnant women. Given that *Escherichia coli* and *Enterococcus faecalis* are components of the biofilm of the intestinal mucosa, have intercellular contact in symbiosis, are partly represented by multidrug-resistant strains, there is an increased risk of complex superinfections. The study of biofilms and the association of bacteria contributes to the development of certain provisions on their antibiotic sensitivity and contributes to a scientifically sound revision of UTI treatment protocols in pregnant women, taking into account the dose of ABD, the behavior of bacteria in biofilms under different drug concentrations and the establishment of antibiotic resistance [10].

THE AIM

The objectives of the study of the spread of antibiotic-resistant strains from the association of *E. coli* - *E. faecalis* in the urogenital tract of pregnant women are to study antibiotic resistance to ABD isolates and to establish differences between antibiotic resistance of *E. coli* and *E. faecalis* isolates under conditions of monostrains and association strains.

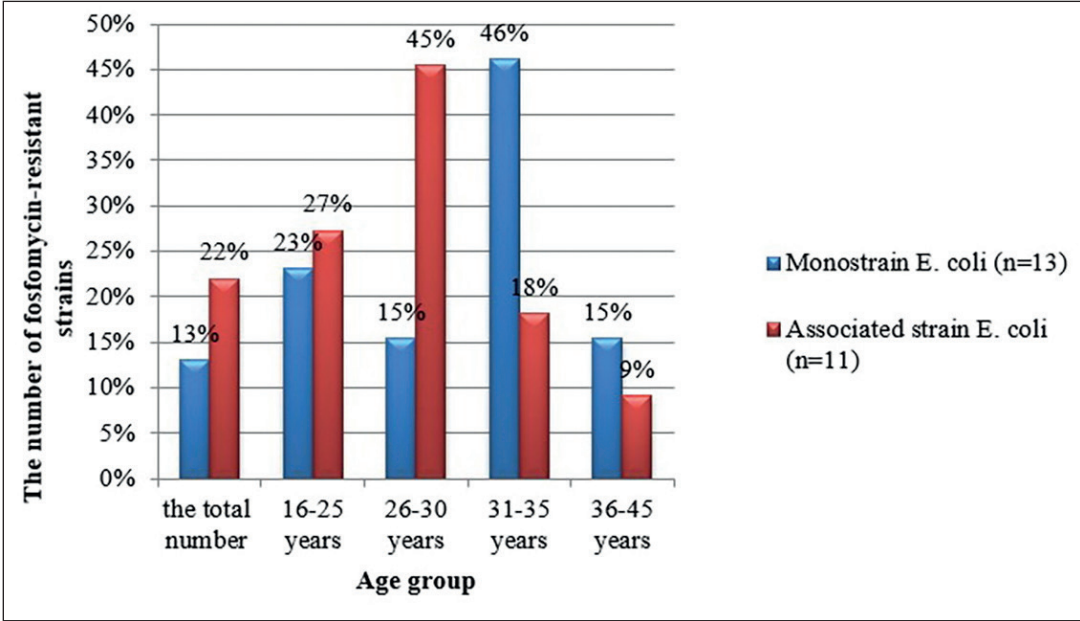


Fig. 1. Frequency of detection of fosfomycin-resistant strains of *E. coli* in women of different ages

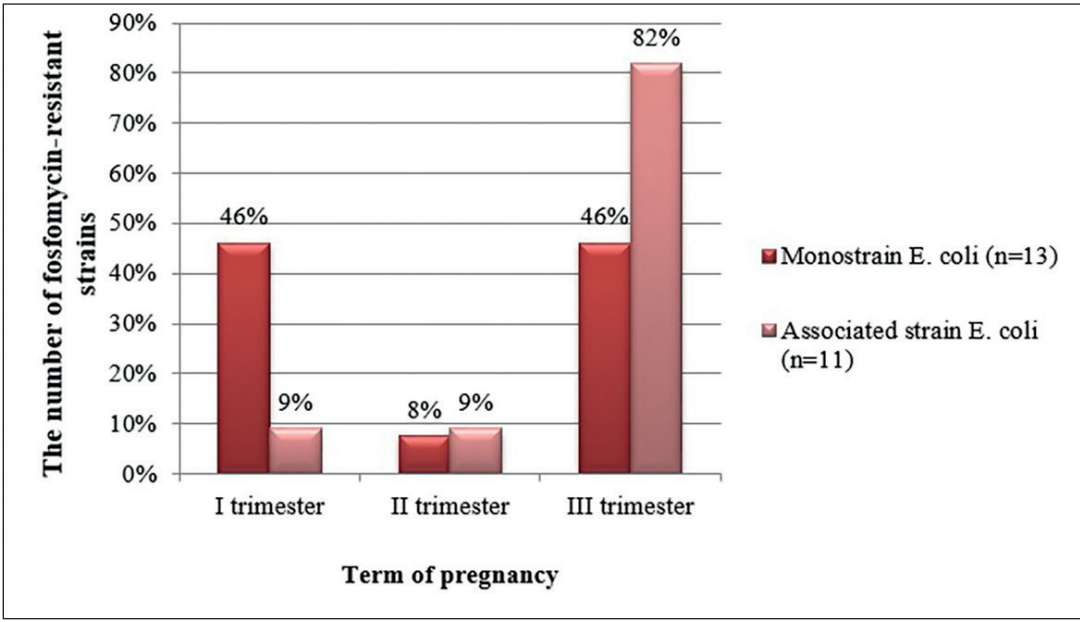


Fig. 2. Frequency of detection of fosfomycin-resistant *E. coli* strains in women of different groups depending on the gestational age

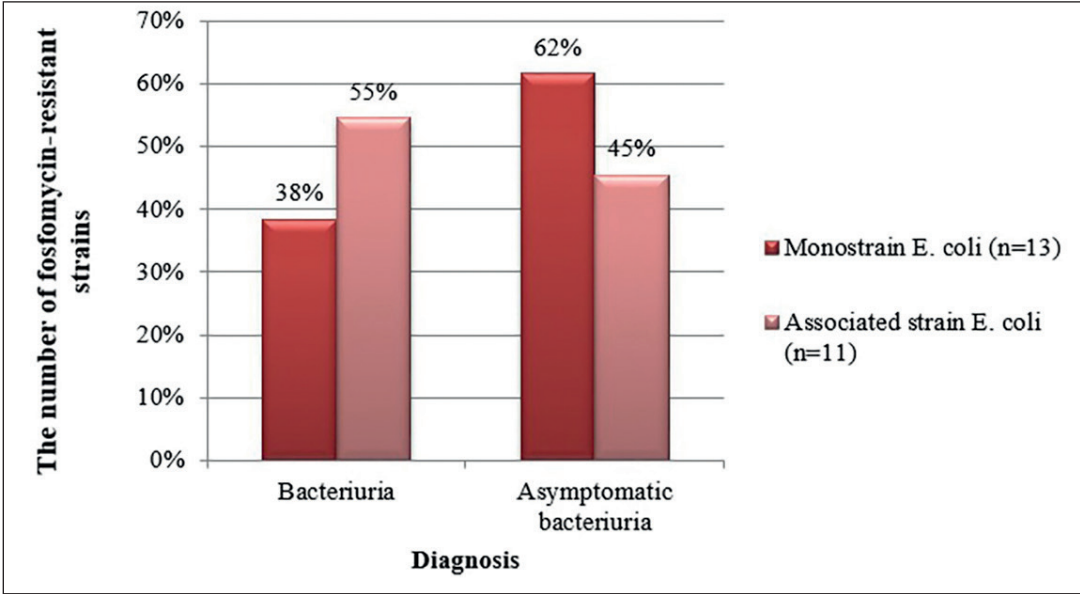


Fig. 3. Frequency of detection of fosfomycin-resistant strains of *E. coli* depending on the diagnosis

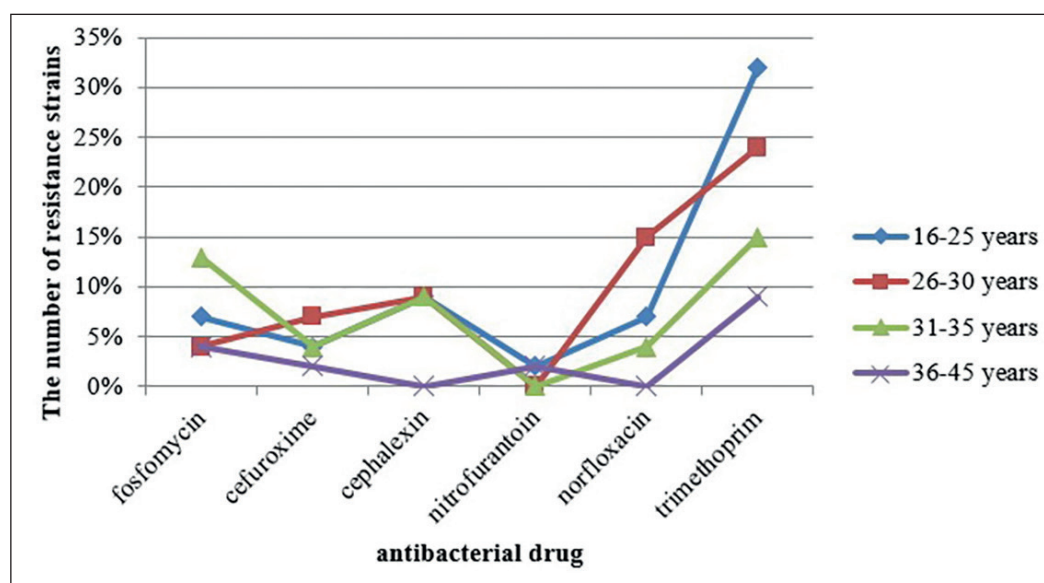


Fig. 4. Frequency of detection of antibiotic-resistant *E. coli* monostrains (n = 45) in different age groups of pregnant women

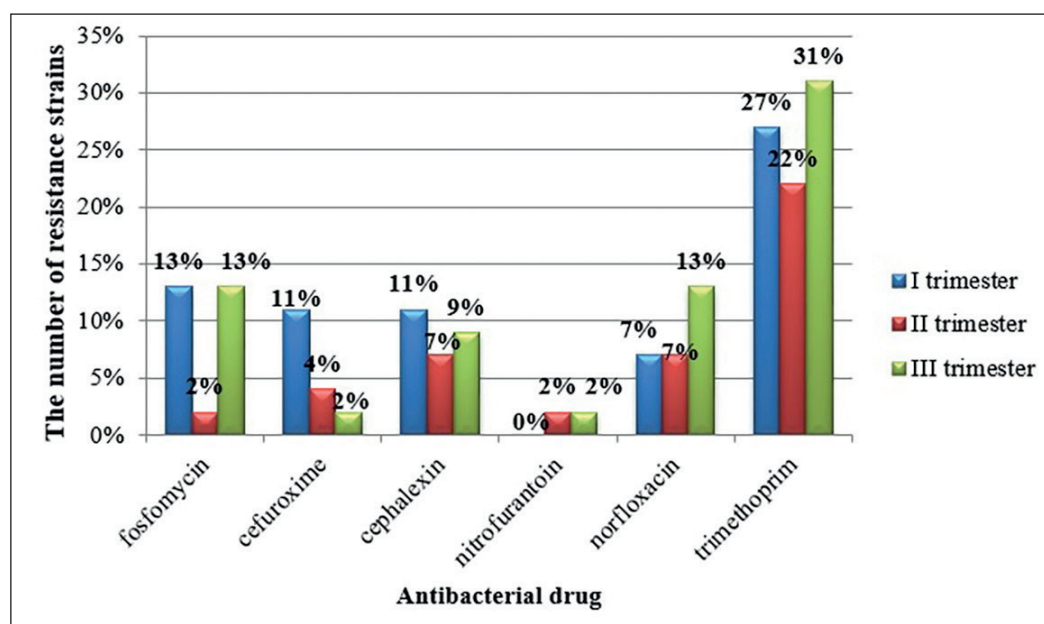


Fig. 5. Frequency of detection of antibiotic-resistant strains of *E. coli* (n = 45) depending on the gestational age

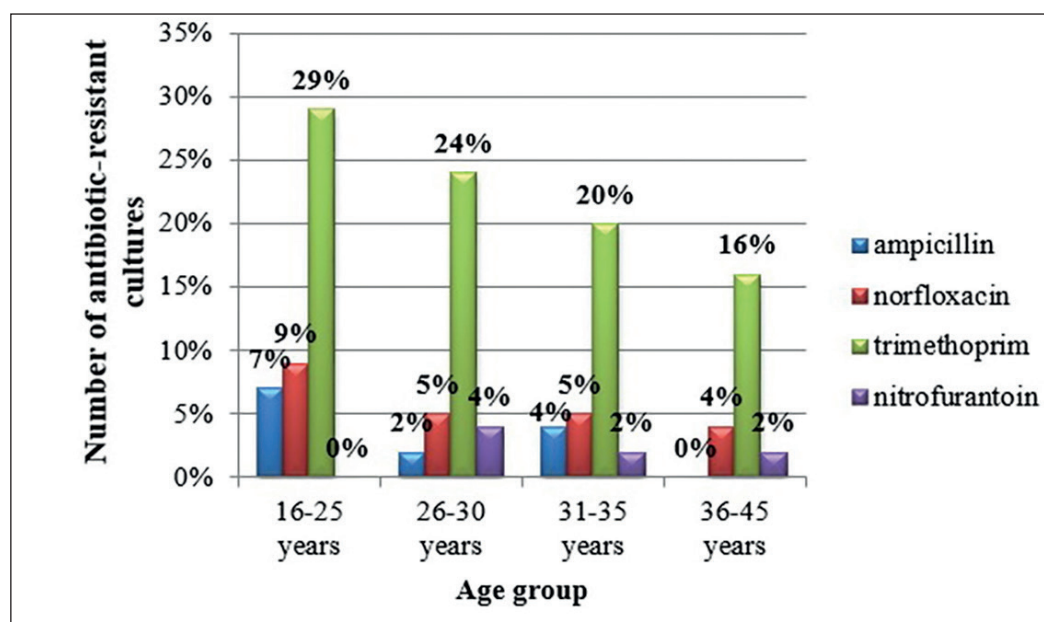


Fig. 6. Frequency of detection of antibiotic-resistant strains of *E. faecalis* (n=55) in different age groups of examined pregnant women

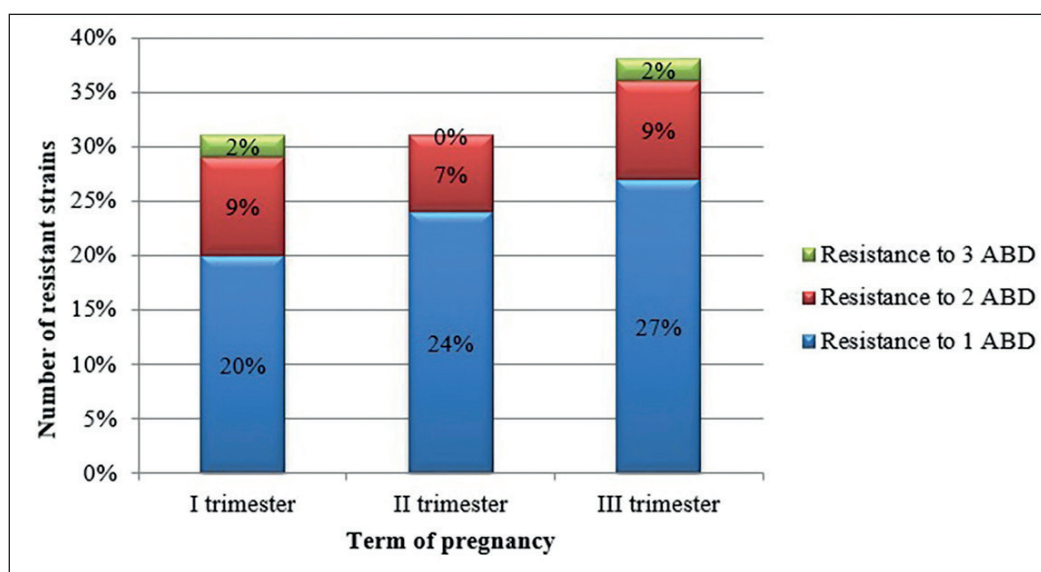


Fig. 7. Frequency of detection of mono- and polyresistant strains of *E. faecalis* (n=55) in women of the examined groups at different stages of pregnancy

MATERIALS AND METHODS

250 urine samples ($v = 100$ ml) were taken from 250 women for the study. Selection was performed in sterile disposable urine containers. Delivery of biomaterial to the laboratory was not more than 2 hours. Primary culture of urine for sterility was performed by the method of sector cultures on agar Columbia with 5% sheep blood (Grasko, Poland) and 0.25% glucose broth. Petri dishes and tubes with plates on lamellar and liquid nutrient media were incubated at 37°C for 18-24 hours. The next day we studied the morphological, tinctorial properties of selected strains, put biochemical tests to identify *E. coli* and *E. faecalis*. Incubation of tubes with nutrient media was performed at 37°C for 18-24 hours. On the third day, the results of biochemical tests were taken into account, the susceptibility of selected strains of microorganisms was determined by the disk-diffusion method in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The results of antibiograms of selected strains of *E. coli* ($n = 100$), *E. faecalis* ($n = 100$) and association *E. coli*-*E. faecalis* ($n = 50$) from the urine of pregnant women were selected for the study. Statistical data processing was performed using ANOVA analysis of variance to find the dependence in the obtained data by studying the significance of differences in averages and correlations.

RESULTS

According to the results of analysis of variance ANOVA growth retardation zones for each antibacterial drug monostain *E. coli* and its strain from the association *E. coli* - *E. faecalis* found a significant difference in the two samples of growth retardation zones to fosfomicin - the criterion of significance (P-value) is <0.05 , which indicates the presence of statistically significant differences. The criterion of reliability of individual samples of growth retardation zones to cefuroxime, cephalexin, nitrofurantoin, norfloxacin and

trimethoprim of two strains of *Escherichia coli* is $P > 0.1$, which indicates the absence of differences in them.

According to the results of analysis of variance ANOVA growth retardation zones for each antibacterial drug mono strain *E. faecalis* and its strain from the association *E. coli*-*E. faecalis*, no differences were found in the respective samples - criterion $P > 0.1$ (for ampicillin, trimethoprim, nitrofurantoin and norfloxacin).

It was found that the number of fosfomicin-resistant mono strains of *E. coli* isolated from the urine of pregnant women is 13 cultures (13% of 100 strains studied), and associated strains of *E. coli* - 11 cultures (22% of the 50 studied strains), which in 1,7 times more.

The number of fosfomicin-resistant strains of *E. coli* depending on the type of culture and age group of pregnant women is presented in Fig. 1.

According to the results of correlation analysis, there is no dependence (coefficient is 0) between the number of fosfomicin-resistant *E. coli* monostains and age, but there is an inverse relationship (coefficient -0.6) between the number of fosfomicin-resistant associated strains of *E. coli* and age that indicates a decrease in the number of such strains depending on the increase in the age category of pregnant women. It was found that among pregnant women aged 16-25 years, the number of fosfomicin-resistant monosteps of *Escherichia coli* isolated from urine is 3 strains (23% of 13 resistant strains), associated *E. coli* - 3 strains (27% of 11 resistant strains). Among pregnant women aged 26-30 years, the number of fosfomicin-resistant monostains of *Escherichia coli* isolated from urine is 2 strains (15% of 13 resistant strains), associated *E. coli* - 5 strains (45% of 11 resistant strains).

Among pregnant women aged 31-35 years, the number of fosfomicin-resistant monostains of *E. coli* isolated from urine is 6 strains (46% of 13 resistant strains), associated with *E. coli* - 2 strains (18% of 11 resistant strains).

Among pregnant women aged 36-45 years, the number of phosphomicin-resistant monostains of *Escherichia coli*

isolated from urine is 2 strains (15% of 13 resistant strains), associated *E. coli* - 1 strain (9% of 11 resistant strains).

Thus, the largest number of fosfomycin-resistant monostrains of *Escherichia coli* (45%) is observed among pregnant women aged 26-30 years, and associated strains of *E. coli* (46%) - among pregnant women aged 31-35 years.

The number of fosfomycin-resistant strains of *E. coli* excreted in the urine, depending on the type of culture and trimester of pregnancy is presented in Fig. 2.

Correlation analysis showed no relationship (coefficient 0) between the number of fosfomycin-resistant *E. coli* monostrains and gestational age, but there is a straightforward relationship (coefficient 0.8) between the number of fosfomycin-resistant *E. coli* strains of pregnancy and trimester pregnancy that is indicating an increase in the number of such strains depending on the increase in gestational age.

It was found that the number of fosfomycin-resistant uropathogenic *Escherichia coli* strains in the first and third trimesters of pregnancy is 6 strains (46% of 13 resistant strains), in the second trimester - 1 strain (8%).

The number of fosfomycin-resistant associated strains of *E. coli* in the first and second trimesters of pregnancy is 1 strain (9% of 11 cultures), in the third trimester - 9 strains (82%).

The number of fosfomycin-resistant strains of *E. coli* isolated from the urine of pregnant women, depending on the type of culture and diagnosis is presented in Fig. 3.

The number of fosfomycin-resistant monostrains of *E. coli* isolated from the urine of pregnant women with bacteriuria is 5 strains (38% of 13 cultures), associated strains - 6 cultures (55% of 11 strains).

The number of fosfomycin-resistant monostrains of *E. coli* isolated from the urine of pregnant women with asymptomatic bacteriuria is 8 strains (62% of 13 cultures), associated strains - 5 cultures (45% of 11 strains).

The distribution of antibiotic-resistant monostrains of *E. coli* depending on the antibacterial drug and age group is presented in Fig. 4

It was found that among fosfomycin-resistant monostrains of *E. coli* ($n = 13$) isolated from the urine of pregnant women, there is no correlation (coefficient - 0.06) between increasing age and the number of resistant isolates to this ABD, of which the largest number - 6 strains (13% of the 45 isolates) were isolated among pregnant women aged 31-35 years.

Among resistant *Escherichia coli* to cefuroxime ($n = 8$), there is a negative correlation (coefficient of -0.6) between the number of such strains and age, namely the number of resistant strains decreases depending on the growth of age among pregnant women.

The correlation between the age of pregnant women and the number of resistant urostrains of *E. coli* to cephalixin ($n = 12$) is -0.7. It was found that among women aged 16-35 years the number of such isolates is distributed evenly (4 strains) between each age group, except for pregnant women aged 36-45 years, among which no resistant to *Escherichia coli* strain was isolated.

The number of *Escherichia coli* resistant to nitrofurantoin is 2 strains isolated from pregnant women of I and IV age groups.

There is a negative correlation between the number of norfloxacin-resistant urostrains of *E. coli* ($n = 12$) and the age of pregnant women, that is the number of resistant strains decreases with age. The largest number of such strains (7 cultures (15% of 45 isolates) was isolated from the urine of pregnant women aged 26-30 years).

Correlation analysis of the data showed that there is a strong negative relationship between the number of trimethoprim-resistant *Escherichia coli* and the age group of pregnant women. The correlation coefficient is -0.9. The number of such strains for women aged 16-25 years is 14 strains (32% of 45 cultures), aged 26-30 years - 11 cultures (24%), aged 31-35 years - 7 strains (15%) and aged 36-45 years - 4 strains (9%).

The distribution of antibiotic-resistant monostrains of *E. coli* depending on the antibacterial drug and the trimester of pregnancy is presented in Fig. 5.

According to the results of correlation analysis, it was found that the number of fosfomycin-resistant monostrains of *E. coli* (coefficient is 0) does not change with increasing gestational age, but from 45 strains 6 isolates (13%) were excreted in the urine of pregnant women in the first and third trimesters.

There was a negative correlation (coefficient of -1) of the number of *Escherichia coli*-resistant strains to cefuroxime and cephalixin depending on the increase in gestational age, namely, the larger the trimester of pregnancy, the less resistant strains to these cephalosporins are released. The number of *E. coli* urostrains resistant to cefuroxime in the first trimester is 5 cultures (11% of 45 resistant isolates), in the second trimester - 2 cultures (4%), in the third trimester - 1 culture (2%).

The correlation between the number of norfloxacin-resistant *Escherichia coli* strains and trimesters of pregnancy was identified as straightforward (coefficient is 1) - the larger the trimester, the greater the number of resistant *E. coli* monostrains (I trimester - 3 strains (7% of the total), III trimester - 6 strains (13%)).

There is a straight line correlation (coefficient is 1) in the number of trimethoprim-resistant *E. coli* monostrains depending on the growth of the gestational age, that is the larger trimester of pregnancy, the more allocated resistant strains to this ABD. The number of resistant to trimethoprim *E. coli* in the first trimester is 12 cultures (27% of 45 resistant isolates), the second trimester - 10 cultures (22%), the third trimester - 14 cultures (31%).

The distribution of antibiotic-resistant urostrains of *E. faecalis* ($n = 55$) depending on the age group of pregnant women and ABD is presented in Fig. 6

According to the results of correlation analysis, an inverse relationship (correlation coefficient is -0.8) was found between the number of ampicillin-resistant *E. faecalis* monostrains and the age of pregnant women, which indicates a decrease in the number of ampicillin-resistant strains depending on age. The number of ampicillin-resistant

E. faecalis isolated from the urine of pregnant women aged 16-25 years is 4 strains (7% of 55 resistant strains), from 26-30 years - 1 strain (2%), from 31-35 years - 2 isolates (4%).

The relationship between the number of norfloxacin-resistant *E. faecalis* urostrains and the age of pregnant women is a reverse linear trend (correlation coefficient is -0.8), indicating a decrease in the number of norfloxacin-resistant enterococcal strains depending on age. The number of norfloxacin-resistant cultures of fecal enterococci isolated from the urine of pregnant women aged 16-25 years is 5 strains (9% of 55 isolates), from 26-30 years - 3 strains (5%), from 31-35 years - 3 strains (5%), from 36-45 years - 2 cultures (4%).

There is a rapid trend and an inverse correlation (coefficient of -0.9) between the number of trimethoprim-resistant *E. faecalis* monostrains and the age of pregnant women, indicating a clear tendency to decrease trimethoprim-resistant enterococcal urostrains depending on the increase in the age of pregnant women. The number of trimethoprim-resistant urocultures of *E. faecalis* isolated from the urine of women aged 16-25 years is 16 strains (29% of 55 resistant isolates), from 26-30 years - 13 strains (24%), from 31-35 years - 11 cultures (20%), from 36-45 years - 9 strains (16%).

There was no correlation (coefficient is -0.3) between the number of resistant strains to nitrofurantoin and the age of pregnant women. The number of resistant to nitrofurantoin strains of *E. faecalis* isolated from the urine of pregnant women aged 26-30 years - 2 strains (4%), from 31-35 years - 1 strain (2%), from 36-45 years - 1 isolate (2%).

The frequency of isolation of antibiotic-resistant monostrains of *E. faecalis* depending on the trimester of pregnancy and the number of ABP to which antibiotic tolerance has been registered is presented in Fig. 7.

According to the results of correlation analysis of the dependence of the number of *E. faecalis* urostrains resistant to 1 ABD ($n = 39$) and the gestational age, a strong straightforward relationship was established (correlation coefficient is 1), which indicates a rapid increase in the number of such strains. Among pregnant women, the number of resistant to 1 ABD enterococci in the first trimester of pregnancy is 11 cultures (20% of 55 isolates), in the second trimester - 13 strains (24%), in the third trimester - 15 strains (27%).

The number of resistant to 2 ABP monostrains of *E. faecalis* ($n = 14$) among pregnant women in the first trimester of pregnancy is 5 cultures (9% of 55 isolates), in the second trimester - 4 strains (7%), in the third trimester - 5 strains (9%).

The number of multidrug-resistant urostrains of *E. faecalis* isolated from pregnant women in the first trimester is 1 culture (2%), in the third trimester - 1 strain (2%).

DISCUSSION

Ramos et al. established that of the 50 strains of *Escherichia coli* isolated from the urine of pregnant women from Sweden, 2 strains (4%) are resistant to cephalixin, 2 strains (4%) - to norfloxacin, 25% - to trimethoprim. The authors

found that from the urine of pregnant women from Uganda, the number of *E. coli* strains resistant to cephalixin is 12 strains (21%), to norfloxacin - 13 strains (23%), to trimethoprim - 49 strains (88%). From the urine of pregnant women in Vietnam, researchers isolated 4 strains (9%) of *Escherichia coli* resistant to cephalixin, 15 strains (36%) to norfloxacin, 29 strains (70%) to trimethoprim [11]. Comparing the obtained data of own research it is established:

- the number of cephalixin-resistant strains of *E. coli* (12 strains) corresponds to the data obtained from women from Vietnam, lower than the number of such strains isolated from women from Uganda, and higher than the number of strains isolated from the urine of women from Sweden;

- the number of *E. coli* strains resistant to norfloxacin (12 strains) is higher than the number of such strains excreted in the urine of pregnant women from Sweden, and lower than the number of norfloxacin-resistant strains of *Escherichia coli* excreted in the urine of women from Uganda and Vietnam;

- the number of trimethoprim-resistant strains of *E. coli* (36 strains) correlates with the obtained data of the authors - resistance of uropathogenic *Escherichia coli* to this antibacterial drug has high values.

Ballesteros-Monrreal et al. found that among pregnant women in Sonora (Mexico) isolated 50 strains of uropathogenic *E. coli*, of which 4% are resistant to fosfomycin, 82% - to cefuroxime, 40% - to nitrofurantoin, 56% - to norfloxacin, 64% - to trimethoprim [12]. In comparison with own data, it is established:

- the number of fosfomycin-resistant *Escherichia coli* ($n = 13$) isolated from the urine of pregnant women in Dnipro is 9% higher than among such a contingent of the state of Sonora (Mexico);

- the number of cefuroxime-resistant growths of *E. coli* is 10 times lower among pregnant women in Dnipro;

- the number of nitrofurantoin-resistant urostrains of *Escherichia coli* is 38% lower than the data obtained among pregnant women in the state of Sonora;

- the number of norfloxacin-resistant urostrains *Escherichia coli* is 44% lower than the results obtained in the state of Sonora;

- the number of trimethoprim-resistant urostrains *E. coli* is 2 times higher, but there is a high resistance to this ABD.

CONCLUSIONS

Thus, the results of the study of antibiotic resistance strains of the association of *E. coli* - *E. faecalis* partially confirmed the hypotheses:

1. The number of resistant to cefuroxime, cephalixin, nitrofurantoin, norfloxacin and trimethoprim uropathogenic strains of *E. coli* does not change depending on the monostep and associated strain of *Escherichia coli* from the urine of pregnant women in Dnipro.
2. It was found that the differences in the number of fosfomycin-resistant uropathogenic strains of *E. coli* depending on the type of culture - the number of fos-

fomycintolerant strains of *Escherichia coli* is 2 times higher among the associated strains, compared with monostrains of the studied microorganism.

3. The amount of uropathogenic strains of *E. faecalis* resistant to ampicillin, trimethoprim, nitrofurantoin and norfloxacin does not change depending on the type of culture isolated from the urine of pregnant women in Dnipro.

Thus, the symbiotic type of existence of two members of the normal intestinal flora on the uroepithelium of pregnant women may be associated with the development of *Escherichia coli* antibiotic resistance only to fosfomycin compared with monoinfection caused by each of these microorganisms.

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ORCID and contributionship:

Olha S. Voronkova: 0000-0003-3380-6871^{A,B,D-F}

Maksym V. Lusta: 0000-0001-9273-6549^{A-D,F}

Yuliia S. Voronkova: 0000-0002-4079-8294^{A,D-F}

Yelyzaveta S. Fawzy: 0000-0001-9973-9638^{A,E,F}

Tetiana H. Ostanina: 0000-0002-1880-5857^{A,C,F}

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olha S. Voronkova

Oles Honchar Dnipro National University
35 Dmytra Yavornitskoho av., 49005 Dnipro, Ukraine
tel: +380962250463
e-mail: voronkova.olga.04@gmail.com

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ORIGINAL ARTICLE

CLINICAL AND NEUROPHYSIOLOGICAL PARALLELS OF THE BRACHIAL PLEXOPATHY IN THE STRUCTURE OF NEUROGENIC THORACIC OUTLET SYNDROME

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Mariia V. Koval, Viktoriia A. Gryb, Viktoriia R. Gerasymchuk, Iryna I. Liskevych

IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT**The aim:** Was assessment of the neurophysiological data and features of clinical picture in patients with neurogenic thoracic outlet syndrome (TOS).**Materials and methods:** 103 patients with upper extremity pain and/or paresthesia or hypotrophy, or a combination of these symptoms were examined. The examination algorithm included: cervical spine radiography, cervical spine and brachial plexuses magnetic resonance imaging (MRI), upper extremity soft tissues and vessels ultrasonic examination, stimulation electroneuromiography with F-waves registration.**Results:** Neurogenic TOS was diagnosed in 29 patients. A significant relationship between the following complaints and neurophysiological parameters was observed: pain, numbness during physical activity and decreased medial antebrachial cutaneous nerve response amplitude by $\geq 25\%$ compared to the contralateral side; hypothenar hypotrophy and decrease of ulnar nerve motor/sensory response amplitude; the 4-5th fingers hypoesthesia and decrease of ulnar nerve sensory response amplitude.**Conclusions:** Medial antebrachial cutaneous nerve amplitudes asymmetry indices of $\geq 25\%$ or lack of response may be considered to be a marker of true neurogenic TOS.**KEY WORDS:** brachial plexopathy, upper extremity pain, thoracic outlet syndrome, neurophysiological diagnostics

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INTRODUCTION

Thoracic outlet syndrome (TOS) involves compression of the subclavian artery, vein, brachial plexus in one of the following sites: spatium interscalenum, spatium costoclaviculare or the space behind the pectoralis minor muscle tendon to the processus coracoideus fixation site [1-3]. It should be noted that the neurovascular bundle lesion at the scalene triangle level is characterized arterial and neurogenic symptoms, because the subclavian vein joins these structures after leaving the interscalene triangle. The term TOS was introduced by R. M. Peet in 1956 and despite the long history of this nosology, its clear diagnostic criteria are not established till nowadays. This situation has arisen probably due to the presence of non-specific complaints, which may be misinterpreted as traumatic, orthopedic or vertebrogenic pathology.

According to compression of vessels and/or nerve fibers TOS is classified into vascular and neurogenic. The prevalence of neurogenic TOS is about 95% among all TOS forms. Neurogenic TOS is therefore divided into true neurogenic and disputed [3-5].

The true neurogenic TOS etiological factors are classified into congenital and acquired. Congenital anomalies are further divided into bone and fibromuscular anomalies [1-6]. A combination of congenital and acquired anomalies is often observed.

The true neurogenic TOS usually manifests with pain, hypoesthesia, paresthesias of localization, typical for the brachial plexus level involvement, gradually progressive atrophies, and is accompanied by pathological changes in instrumental examination

methods findings, and medical history of cervical and thoracic injuries [4, 7-9]. Clinical picture of disputed TOS is similar to the true neurogenic one, but there are no pathognomonic changes found in additional examination methods results.

THE AIM

The aim of study was to analyze the clinical and neurophysiological features of brachial plexopathy in the neurogenic TOS structure in order to formulate the diagnostic algorithm.

MATERIALS AND METHODS

The study was performed on the basis of the neurology and neurosurgery department of Ivano-Frankivsk National Medical University during 2017 and the Research and Practical Center of neurophysiological studies during 2018-2020. Totally there were 103 patients examined with upper extremity pain and/or paresthesia or hypotrophy, or a combination of these symptoms. The patients' mean age of was 37.39 ± 5.17 years, 61 (63%) of them were females.

The algorithm of the planned examination included: cervical spine radiography, cervical spine and brachial plexuses magnetic resonance imaging (MRI) (Siemens 1.5 T), upper extremity soft tissues and vessels ultrasonic examination, stimulation electroneuromiography (ENMG) with F-waves registration (Neurosoft, Neuro EMG-mi-

cro, 2004). Doppler ultrasonography was performed in the position with the upper extremity lowered along the torso and during the Adson's, Eden's, Wright's and EAST provocation tests.

Exclusion criteria:

- cervical spine discoradicular conflict, myelopathy, syringomyelia (verified by MRI);
- tunnel neuropathy, polyneuropathies (diagnosed by ENMG);
- traumatic/orthopedic pathology (verified after orthopedist consultation with the radiological examination (X-ray / MRI and ultrasonic study of the musculoskeletal system);
- upper extremities blood vessels thrombosis (according to ultrasonic examination data);
- neoplastic and postradiation lesion of the chest and upper extremity girdle.

After the examination and data comparison with the inclusion/exclusion criteria 29 patients remained under our supervision. Statistical processing of the study results was performed with the use of computer with a software environment for statistical calculations " R " (R Core Team RR . A language and environment for statistical computing. R Foundation for statistical computing [Internet]. 2018. Available from: URL: [https:// www.r-project.org/](https://www.r-project.org/)).

RESULTS

Complaints on pain occurred in 21 (72.4%) cases; pain was localized in the rhomboid muscles, anterior chest surface, neck area, brachioscapular area, forearm, hand.

The abovementioned complaints mostly corresponded to the dominant arm, which was observed in 17 (80.9%) patients. In addition, the pain which occurred during physical activity, persisted also after the termination of activity.

26 (89.6%) patients complained on numbness, which in 4 (15.4%) cases was permanent, in 4 (15.4%) cases it was positional, in 5 (19.2%) patients numbness occurred during physical activity, 6 (23.1%) patients experienced it during nighttime, and 7 (26.9%) patients noted a combination of complaints on numbness in certain position and during nighttime.

The feeling upper extremity weakness was noted by 17 (58.6%) patients; 7 (41.1%) of them complained on the 4th-5th fingers, 4 (23.5%) patients felt it in the entire hand, in 6 (35.3%) cases it involved all muscles of the upper extremity. In order to objectify the complaints, the muscle strength of the upper extremity was assessed; the strength decrease was detected in 4 (23.5%) cases, namely in m. abductor pollicis brevis and m. interosseus dorsalis I.

Complaints on hand fine motor skills impairment were observed in 13 (44.8%) cases, and were objectively detected in 3 (10.3%) patients. The feeling of hand discomfort bothered 5 (17.2%) patients.

In neurological status hypothenar hypotrophy was observed in 2 (6.8%) cases; in 1 (3.4%) patient thenar, hypothenar and interosseous muscles hypotrophy were observed. Triceps hyporeflexia on the symptomatic side was observed in 1 (3.4%) patient.

Skin hypoesthesia in the 4th-5th fingers along was determined in 4 (13.8%) patients; in 3 (10.3%) cases it included the 4th-5th fingers and the ulnar aspect of forearm.

Supraclavicular region palpation was painful in 5 (17.2%) patients, and in 2 (6.9%) cases it was accompanied by numbness in comparison with the asymptomatic extremity. Intrescalene trigon palpation elicited pain in 4 (13.7%) cases, palpation of the pectoralis minor muscle projection site was painful in 1 (3.4%) patient.

Raynaud's syndrome was observed in 6 (20.6%) cases; it was characterized by presence of symptoms only on the symptomatic side, which differs from the primary Raynaud's syndrome. The majority of authors tend to think that Raynaud's syndrome is a manifestation of the vascular TOS variant [7]. Due to exclusion of the vascular TOS from our study it may be considered to be its secondary variant caused by irritation of the brachial plexus sympathetic fibers. R. T. Alekperov (2014) mentioned the possibility of the primary Raynaud's syndrome misdiagnosis in case of TOS [10], which leads to inadequate treatment tactics.

Bone abnormalities were radiologically diagnosed. In 3 (10.4%) patients an additional cervical rib was detected; in 2 (6.9%) cases the C7 transverse process elongation up to 20 mm and 23 mm (N=13-17 mm) was found out; in 1 (3.4%) patient the costoclavicular space narrowing due to the formation excessive osteocallus formation after clavicular injury was detected. Gruber (1869) suggested classification of cervical ribs into 4 groups, where the 3rd and 4th groups includes the cervical rib presence with the fixed fibrous cords, which are radiologically negative.

MRI revealed fibrous cord in the interscalene triangle in 1 (3.4%) person. V. K. Singh (2014) after evaluation of the MRI preoperative findings and postoperative results indicates that the sensitivity of this method is 41%, specificity is 33%.

In order to assess the neurophysiological examination data, the study included 20 apparently healthy individuals.

The neurophysiological investigation results are presented in table I; the parameters of the motor responses, the F - wave latency, the sensory responses amplitude and latency were studied.

The most informative parameters during the neurophysiological data comparison of patients with the upper extremity pain and AHI were the MACN indicators. All patients who were included into the study had unilateral complaints, so we decided to compare the obtained results with the same data of asymptomatic limb (Table II).

During evaluation of the medial antebrachial cutaneous nerve response amplitude in 5 patients the amplitude difference between two sides was $\geq 25\%$; in 3 of them the response could not be registered.

Data of significant relation between the complaints and ENMG parameters are presented in Table III.

After assessment of patients' complaints, disease history, neurological status and of instrumental examination methods results, the true neurogenic TOS was diagnosed in 13 (44.8%) patients, the disputed neurogenic TOS was diagnosed in 16 (55.2%) patients. There were no changes detected in ENMG in patients with disputed neurogenic TOS. In our opinion, the lack of neurophysiological changes in patients with disput-

Table I. ENMG parameters of patients with the neurogenic TOS and apparently healthy individuals

ENMG parameters	UN		MN		MACN	
	AHI	TOS	AHI	TOS	AHI	TOS
Motor response amplitude, μ V	9.83 \pm 2.71	6.48 \pm 1.57	10.69 \pm 2.64	8.32 \pm 2.86	-	-
Sensory response amplitude, μ V	7.36 \pm 3.04	6.09 \pm 3.62	10.53 \pm 2.09	8.63 \pm 2.47	18.7 \pm 4.91	6.52 \pm 3.84
Sensory response latency, ms	2.29 \pm 0.3 3	2.11 \pm 0.67	1.82 \pm 0.78	2.05 \pm 0.26	2.13 \pm 0.28	2.68 \pm 0.48
F-wave latency, ms	28.91 \pm 1.32	29.61 \pm 4.78	27.01 \pm 2.78	28.13 \pm 1.64	-	-

Note: UN - ulnar nerve; MN - median nerve; MACN - medial antebrachial cutaneous nerve; AHI - apparently healthy individuals; TOS - patients with TOS.

Table II. ENMG parameters of medial antebrachial cutaneous nerve of symptomatic and asymptomatic upper extremity

N patient	Sensory response amplitude (μ V)		Sensory response latency, ms	
	Symptomatic arm	Asymptomatic arm	Symptomatic arm	Asymptomatic arm
1	14	30	3.1	2.8
2	0	18	-	2.2
3	0	21	-	1.8
4	11	24	2.2	2.1
5	5	19	2.9	1.6
6	0	14	-	1.9
7	8	31	3.0	2.9
8	17	35	2.4	2.4

Table III. Significance of relation between the complaints and ENMG parameters

		Q	X ²	P
1.	Pain intensification/occurrence after physical activity the difference of the MACN response amplitudes D/S \geq 25 %	0.54	2.8	<0.05
2.	Hypoesthesia of the 4-5 fingers the amplitude of the UN sensory response	0.54	2.8	<0.05
3.	Numbness intensification/occurrence after physical activity the difference of the MACN response amplitudes D/S \geq 25 %	-0.71	3.0	<0.05
4.	Hypothenar hypotrophy the amplitude of the UN motor response	-0.71	3.0	<0.05
5.	Hypothenar hypotrophy the amplitude of the UN sensory response	-0.53	1.1	<0.05
6.	Paresthesia of the ulnar aspect of forearm and hand the difference of the MACN response amplitudes D/S \geq 25 %	-0.51	3.8	<0.05

Note: UN - ulnar nerve; MACN - medial antebrachial cutaneous nerve.

ed neurogenic TOS may be associated with technical difficulties in the brachial plexus stimulation in the supraclavicular area, which leads to difficulties in diagnosing local demyelination / conduction block, especially in case of excessive body weight and / or Kovtunovych's pseudotumor. In some patients with controversial neurogenic TOS complaints occurred only at a certain limb position or at certain movements and regressed after position changing, so apparently they were accompanied by a short-term nerve fiber compression and therefore absence of neurophysiological abnormalities.

Neurophysiological changes in patients with true neurogenic TOS were observed in 8 (61.5%) cases. A significant difference of the medial antebrachial cutaneous nerve sensory response between the symptomatic and asymptomatic extremities was observed in 87.8% cases; decrease by \geq 25% compared to

the asymptomatic limb or lack of response were considered to be pathological.

DISCUSSION

Serror (2002) was the first to suggest that the difference of the medial antebrachial cutaneous nerve amplitude response of more than 50% should be considered abnormal [12]. B. E Tsao et al. (2014) provided data of a retrospective review of the neurophysiological parameters in patients with surgically verified true neurogenic TOS, where the difference of the sensory response amplitude was assessed, and amplitude of less than 50% compared to the contralateral limb was regarded as abnormal [11].

The role of neurophysiological studies in the diagnosis of neurogenic TOS is quite controversial. R. Rous-

seff et al. (2005) describe 20 surgically verified cases of neurovascular compression in the thoracic aperture, but differences from the normal neurophysiological parameters were determined in only 2 patients. B. Machanic (2008) described his own observation where 41 patients with neurogenic TOS were undergoing the ENMG examination and in 40 of them deviations from the normal parameters were observed; the latency, the medial antebrachial cutaneous nerve amplitude asymmetry of more than 50%, stimulation of C8 spinal root were assessed. Later all these patients underwent surgical treatment of the compression at the interscalene trigon level; diagnosis was confirmed intraoperatively in all patients. After surgery regression of all symptoms, increase of amplitude and decrease of latency of the medial antebrachial cutaneous nerve were observed in all of the patients.

The sensitivity of determination of the medial antebrachial cutaneous nerve amplitudes asymmetries in patients with true neurogenic TOS of $\geq 25\%$ or lack of response, which we obtained in our study, is 33.33%; its specificity is 83.33%, test accuracy is 64.86%, the prognostic value of a positive test result (PVP) is 66.66%, and the prognostic value of a negative test result (PVN) is 64%.

CONCLUSIONS

According to the literature data and the results of our study, the most sensitive neurophysiological parameters in the diagnosis of the true neurogenic TOS are the medial antebrachial cutaneous nerve amplitude with the following results evaluation in comparison with the asymptomatic limb. During assessment of the medial antebrachial cutaneous nerve amplitudes asymmetry the difference of $\geq 25\%$ or lack of response should be taken into account. According to the results of our study, the sensitivity of determination of the medial antebrachial cutaneous nerve amplitudes asymmetries in patients with the true neurogenic TOS of $\geq 25\%$ or lack of response, which we obtained in our study, is 33.33%; its specificity is 83.33%, test accuracy is 64.86%, which demonstrates the feasibility of the study in patients with possible neurogenic TOS.

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ORCID and contributionship:

Mariia V. Koval: 0000-0002-4617-0828^{A,D}

Viktoriia A. Gryb: 0000-0001-6111-7921^{E,F}

Viktoriia R. Gerasymchuk: 0000-0001-8481-8253^{A,C,D}

Iryna I. Liskevych: 0000-0003-3957-7176^{B,C}

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mariia V. Koval

Ivano-Frankivsk National Medical University
2 Halytska St., 76018 Ivano-Frankivsk, Ukraine
tel: +380969520355
e-mail: mkoval2904@gmail.com

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ORIGINAL ARTICLE

NEW POSSIBILITIES FOR MODIFYING THE COURSE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: THE EFFECT OF TIOTROPIUM BROMIDE ON CERTAIN PATHOGENETIC LINKS OF NEOCOLLAGENOGENESIS AND LOCAL IMMUNE DEFENCE OF THE BRONCHIAL TREE

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Mykola M. Ostrovskyy¹, Mariana O. Kulynych-Miskiv¹, Khrystyna I. Volnytska², Lilia Dm. Todoriko², Kseniia M. Ostrovska¹, Oleksandr I. Varunkiv¹, Iryna O. Savelikhina¹

¹IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

²DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

³BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

The aim: To evaluate the dynamics of the interferon and collagen-IV systems in bronchoalveolar lavage in the treatment of chronic obstructive pulmonary disease using the tiotropium bromide medication.

Materials and methods: The study involved 60 COPD patients with bronchial obstruction of the II degree before and on days 30 and 60 of therapy using conventional treatment regimens and inhalations of tiotropium bromide at the dose of 18 mcg once a day. The collagen-IV levels in bronchoalveolar fluid were determined by means of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "Biotrin Collagen IV EIA" reagents. The level of IFN- γ was identified with the help of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "ProKon" reagents (LLC "Protein Contour", Russia) in bronchoalveolar fluid obtained during fiber-optic bronchoscopy.

Results: When examining Group I patients on the 30th day we found out that the content of collagen-IV in the bronchoalveolar fluid had decreased by only 10.29% ($p < 0.05$). Detection of collagen-IV indices in Group II patients on the 30th day of tiotropium bromide use showed the 29.43% ($p < 0.05$) decrease in its content as compared to the initial indices. In Group III patients, the concentration of collagen-IV had a maximum tendency to normalize and made up (24.72 ± 1.15) ng/ml, and decreased by 2.44 times ($p < 0.05$) as compared to the initial indices. Our examination of 12 patients from the comparison group I on the 60th day of treatment revealed even a slight increase in the content of collagen-IV in the bronchoalveolar fluid, as compared with the data obtained on the 30th day. The identified IFN- γ deficiency is indicative for the COPD of the II degree of bronchial obstruction, and its indices were 2.29 times lower than those observed in people from the control group. On day 30, we found out that the content of IFN- γ in Group I patients increased by only 10.29% ($p > 0.05$). Detection of IFN- γ in Group II patients showed 42.27% ($p < 0.05$) increase in its content as compared to the initial indices. The most favorable dynamics of IFN- γ levels in bronchoalveolar contents was observed in Group III patients, and at the time of observation it made up (1.16 ± 0.08) pg/ml, having 2 times ($p < 0.05$) increased as compared to the initial indices. However, in contrast to those taking tiotropium bromide, we examined 12 patients from Group I on the 60th day of treatment and found no significant positive dynamics of IFN- γ content in bronchoalveolar fluid as compared to the indices obtained on day 30.

Conclusions: The obtained findings indicate the effect of tiotropium bromide on the reduction of interferon- γ and reduce of collagen-IV levels, which depend on the duration of its use.

KEY WORDS: chronic obstructive pulmonary disease, tiotropium bromide, collagen-IV, IFN- γ

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is one of the most serious diseases in terms of disability and economic costs, the second most common infectious disease in the world, ranks fourth in the structure of mortality and is characterized by the persisted tendency for its constantly increasing prevalence rates due to the spread of bad habits on the one hand and extending life expectancy on the other [1, 2]. In the "European Lung White Book" Ukraine is presented as one of the countries with the highest mortality rate due to respiratory pathologies among men [3].

During the period of 2015–2016, the incidence of chronic bronchitis in Ukraine has increased by 0.51%, which puts this pathology in the category of strategic issues of domestic medicine [4]. Despite the fact that the development of treatment methods for COPD is well financed, it still continues to be one of the leading causes of death in the world.

The course of COPD becomes especially aggressive in the II degree of bronchial obstruction with the onset of morphological changes of the airways (sclerosis, fibrosis, bronchospasm, edema, hypercrinia, dyscrinia), resulting in bronchial remodeling and subsequent galloping progres-

sion of its systemic manifestations. Respiratory remodeling is a pathological process observed in chronic inflammatory and obstructive respiratory diseases [5].

COPD is characterized by asymptomatic or paucisymptomatic onset of the disease with subsequent progressive increase in the severity of the condition, as well as steadily progressive reduction of the pulmonary function (PF), which is the most specific and prognostic sign of the disease. Unfortunately, this pathology is often diagnosed at later stages, when the most advanced treatment programs are not able to slow down the steady progression of the disease.

The UPLIFT (Understanding Potential Long-term Impacts on Function with Tiotropium) findings show that selective M3 cholinolytic tiotropium has demonstrated a long-term improvement of the lung function in the four-year treatment of COPD patients [6]. While studying the group of patients who were administered tiotropium it became possible to observe the decrease of the mortality risk by 16 % ($p = 0.016$), positive effect of such treatment on survival rate ($p = 0.034$), prevention of disease exacerbation ($p < 0.001$) and significant decrease in the COPD destabilization risk, requiring further hospitalization (risk ratio 0.86; $p < 0.002$) as compared with the control group [6]. Additionally, large-scale clinical studies suggest that the use of long-acting muscarinic antagonist tiotropium in the treatment of COPD patients delays airway deterioration in dynamics up to 4 years, which should probably contribute to the involution of morphological changes in the bronchi. [6-9].

In our opinion, the effectiveness of treatment of any pathology depends on the deep knowledge of all stages of its pathogenesis. Therefore, the study of complex and multisystem processes that occur in the body in COPD, against the background of the therapeutic standards with the inclusion of tiotropium bromide medication with its characteristic effects, is quite relevant and will contribute to modern science and understanding of the need for a long-term basic treatment by both patients and healthcare professionals.

THE AIM

The goal of this study is to evaluate the dynamics of the interferon and collagen-IV systems in bronchoalveolar lavage in the treatment of chronic obstructive pulmonary disease using the tiotropium bromide medication.

MATERIALS AND METHODS

The study involved the examination of 60 patients with COPD II degree of bronchial obstruction. The diagnosis was verified and formulated in accordance with the order №555 of the Ministry of Health of Ukraine from June 27, 2013 "On approval and introduction of medical and technological documents for standardization of care in chronic obstructive pulmonary disease" [1, 10]. The investigation was performed before the start of treatment and on days 30

and 60 of therapy which involved the use of conventional treatment regimens and the administration of tiotropium bromide by inhalation of 18 mcg, once a day.

In order to study the effectiveness of the suggested treatment programs for COPD II degree of bronchial obstruction, all patients were divided into groups depending on the prescribed treatment.

Group I – 20 patients who did not receive the selective M3 long-acting cholinolytic in their comprehensive therapy [1];

Group II – 40 patients who were prescribed a 30-day course of tiotropium bromide on the background of comprehensive treatment of the exacerbation phase.

Group III – 21 patients who were prescribed a 60-day course of tiotropium bromide on the background of comprehensive treatment of the exacerbation phase.

The control group consisted of 15 apparently healthy individuals (AHI) without any signs of diseases of the respiratory tract and other pathologies of the internal organs.

The material of study was bronchoalveolar lavage, obtained considering the localization of inflammatory lesions of the lungs during fiber-optic bronchoscopy by Clements (1967) method in modification of Ramires (1980). The collagen-IV levels in bronchoalveolar fluid were determined by means of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "Biotrin Collagen IV EIA" reagents. The level of IFN- γ was identified with the help of enzyme-linked immunoassay using "StatFax 303 Plus" analyzer and "ProKon" reagents (LLC "Protein Contour", Russia) in bronchoalveolar fluid obtained during fiber-optic bronchoscopy.

RESULTS

The analysis of findings, obtained by fiber-optic bronchoscopy that was performed to 60 patients with COPD II degree of bronchial obstruction before the start of treatment, showed that mostly identified signs were: severe catarrhal inflammation – in 48.8% of patients and purulent inflammation – in 28.8% of the examined individuals. Occasionally, moderate catarrhal inflammation was observed in 12.8% of patients, and 6.4% of the examined individuals had atrophy of the bronchial mucosa; the structure of bronchial mucosa was close to normal – in 3.2% of patients. Repeated fiber-optic bronchoscopy, which was performed to 20 patients from Group I on the 30th day of treatment, showed mainly ($p < 0.05$) moderate catarrhal inflammation in 60.0% of patients, while the marked form of catarrhal inflammation was observed in 40.0% of cases.

Particularly, we would like to emphasize the obtained findings of fiber-optic bronchoscopy performed to 20 patients from Group II on the 30th day of treatment. We revealed a significant decrease ($p < 0.05$) in the proportion of severe catarrhal inflammation by 31.3% in patients from Group II, which was 1.9 times lower than among patients receiving basic treatment regimens. However, the proportion of moderate catarrhal inflammation has increased to 68.8% ($p < 0.05$) among Group II patients. At the same

Table I. Indices of collagen-IV (ng/ml) in bronchoalveolar fluid of patients with chronic obstructive pulmonary disease II degree of bronchial obstruction during treatment with tiotropium bromide

Study group	Indices of collagen-IV in bronchoalveolar fluid			p
	before treatment	on day 30 of treatment	on day 60 of treatment	
I, n=20	60.21±1.08	55.21±1.12	57.08±1.27	< 0.05
II, n=40	62.86±1.25	44.36±1.19		< 0.05
III, n=21	59.07±1.33		24.72±1.15	< 0.05
AHI, n=15	9.87±0.52			

Table II. Indices of IFN-γ (pg/ml) in bronchoalveolar fluid of patients with chronic obstructive pulmonary disease II degree of bronchial obstruction during treatment with tiotropium bromide

Study group	Indices of IFN-γ in bronchoalveolar fluid			p
	before treatment	on day 30 of treatment	on day 60 of treatment	
I, n=20	0.59±0.06	0.68±0.12	0.71±0.07	> 0.05
II, n=40	0.56±0.05	0.97±0.07		< 0.05
III, n=21	0.58±0.03		1.16±0.08	< 0.05
AHI, n=15	1.28±0.11			

time, fiber-optic bronchoscopy of Group III patients on day 60 from the start of treatment, showed that the proportion of moderate catarrhal inflammation increased to 82.6% ($p < 0.05$), and the proportion of marked catarrhal inflammation made up only – 17.4% ($p < 0.05$). Thus, the obtained findings indicate higher effectiveness of comprehensive treatment of patients with COPD II degree of bronchial obstruction, who were administered tiotropium bromide medication as compared to the patients whose treatment regimens did not include this medication. The use of tiotropium bromide medication in the treatment of patients with COPD II degree of bronchial obstruction has also resulted in faster regression of clinical symptoms of the pathology, positive changes in the data of spirometry and laboratory dynamics.

The studies have revealed that the collagen-IV level in bronchoalveolar lavage of healthy individuals was: (9.87 ± 0.52) ng/ml, and IFN-γ (1.28 ± 0.11) pg/ml. On admission of patients with COPD II degree of bronchial obstruction, there was a significant increase in levels of collagen-IV in bronchoalveolar fluid – up to (60.71 ± 1.18) ng/ml ($p < 0.05$) on the background of IFN-γ depression to (0.56 ± 0.07) pg/ml ($p < 0.05$).

The examination of Group I patients on the 30th day of treatment, we found out that the content of collagen-IV in bronchoalveolar fluid decreased by only 10.29% ($p < 0.05$). Detection of collagen-IV levels in Group II patients with COPD II degree of bronchial obstruction on the 30th day of tiotropium bromide use showed the decrease in its content by 29.43% ($p < 0.05$). A real qualitative breakthrough in the dynamics of collagen-IV levels in bronchoalveolar lavage was observed in Group III patients, who received tiotropium bromide for 60 days. At the end of observation, the concentration of collagen-IV in bronchoalveolar fluid had maximum tendency to normalize and made up (24.72 ± 1.15) ng/ml, that was 2.44 times ($p < 0.05$) lower than the indices observed in group with baseline therapy regimens. The examination of 12 patients from the comparison group I on day 60 revealed even a slight increase in collagen-IV

levels in bronchoalveolar fluid, as compared to the data obtained on day 30 (Table I).

Deficiency of IFN-γ in bronchoalveolar fluid of patients with COPD II degree of bronchial obstruction is indicative and its indices were 2.29 times lower as compared to those observed in the control group (Table II). On day 30, we found out that the content of IFN-γ in bronchoalveolar fluid of Group I patients increased only by 10.29% ($p > 0.05$). Detection of IFN-γ indices in Group II patients with COPD II degree of bronchial obstruction on the 30th day of tiotropium bromide use showed an increase in its content by 42.27% ($p < 0.05$), as compared to the indices observed with the use of baseline therapy regimens. As in case of collagen-IV levels, the dynamics of IFN-γ levels in bronchoalveolar fluid was the most favorable in Group III patients who received tiotropium bromide for 60 days. At the end of observation, the concentration of IFN-γ in bronchoalveolar fluid had the maximum tendency to normalize and was (1.16 ± 0.08) pg/ml, and was 2 times ($p < 0.05$) lower as compared to the indices observed with the use of baseline therapy. However, in contrast to those taking tiotropium bromide, we examined 12 patients from Group I on day 60 and found out no significant positive dynamics of IFN-γ in bronchoalveolar fluid, as compared to the data obtained on day 30 (Table II).

Tendency for regeneration of local IFN-γ levels in patients with COPD II degree of bronchial obstruction treated with tiotropium bromide is worth special attention, since it substantiates the whole cascade of sanogenetic mechanisms for resolving pathological changes in the inflammatory process of the bronchial tree. Currently, interferons are isolated into a special class of cytokines, which were previously considered exclusively as antiviral factors, but afterwards their antitumor and immunomodulatory activities were discovered. [11]. The antibacterial property of interferons is associated with their increased phagocytic activity, increased production of immunoglobulins and increased cytotoxicity of natural killer cells [11]. The interferon system has neither specialized cells

nor specialized organs, however, the biggest producers of interferon are immunocompetent cells. When cells are stimulated by an inducer (infectious agent), the genes encoding interferon proteins are activated and the production-translation of these proteins occurs. [11]. Thus, cells start producing interferon, which, on the one hand, inhibits the proliferation of infectious agents, and on the other – enhances the expression of molecules of the major histocompatibility complex class I on the surface of altered cells and causes activation of NK cells, which initiate cytolysis of damaged cells and produce IFN- γ , which directs the development of the immune response by T-helper-1 type [11]. Macrophages, activated by the contact with antigen and IFN- γ , produce IL-12, which stimulates the differentiation of immature CD4 lymphocytes into type 1 T-helpers, which after antigen presentation are activated and produce a number of cytokines (IL-2, TNF- α , IFN- γ) [11]. The effect of these transmitters is the activation of cytotoxic CD8 lymphocytes, which provide cytolysis of the corresponding target cells, culminating in the elimination of pathogens and sanation of the inflammatory process in the bronchi [11].

DISCUSSION

The study has also showed the reduction in collagen-IV content in bronchoalveolar fluid in patients with COPD II degree of bronchial obstruction which were treated with tiotropium bromide. In order to understand the significance of this effect, it is necessary to focus on the role and localization of collagen in the human body. It is a well-known fact that a special layer is visualized between the epithelial membrane and the underlying connective tissue, it is located directly at the base of the epithelial cells and is called the basement membrane. [11]. Typical basement membrane (40 - 120 nm thick) consists of two layers of different origin [12-14]. The first layer of the basement membrane is the basal plate – a separate homogeneous electron-dense layer with a thickness of 50-100 nm, which is located just above the “felt-like” network of reticular fibers, which actually form the second layer [13]. It has been established that the basal plate always follows the contours of the basal surface of epithelium and the distance between them makes up 40 nm [14]. Basal plates may be connected not only with epithelium but also with nerve and muscle fibers [13].

The basement membrane performs two main functions: an elastic framework and a barrier for filtration and diffusion of substances (low molecular weight compounds, intercellular fluid) [13]. It is characterized by a unique form of collagen – type IV. These structures are synthesized by fibroblasts (Latin “fibra-” meaning the fiber, and Greek “blastos-” meaning the embryo). In addition, fibroblast-related cells such as osteoclasts and chondroblasts are also capable of synthesizing collagen. [14].

Currently, ten types of collagen are identified, and they are different in composition of α -chains and a number of functions. The most studied are: Type I – connective tissue

proper (loose and dense) and bone tissue, Type II – hyaline cartilage, Type III – skin of the fetus, arteries, Type IV – basement membrane, Types V - VI collagen is involved in ossification, chondroprotection. Types IX - X may play a role in the maturation of connective tissue proper [12-14].

The three α -chains are identical in all types of collagen, except for Type I [14]. However, the collagen of the basement membrane (type IV) contains much more carbohydrate side chains, as well as more hydroxylysine and hydroxyproline [13]. The presence of hydroxylysine ensures the stability of collagen fibers of the basement membrane, due to the cross-linking of collagen molecules. The hydroxylysine molecule promotes the attachment of short carbohydrate residues – galactose and glucose.

Collagen IV is synthesized in the form of a precursor – procollagen. Synthesis of α -chains of procollagen occurs by means of polyribosomes of the granular endoplasmic reticulum [14]. The synthesized chains are 13 nm longer. A certain number of included proline and lysine residues are hydroxylated within 3 min in the synthesized chain, while the synthesis of the whole chain lasts from 5 to 6 min. The residual tail regions of α -chains are cleaved by means of peptidase enzyme on the cell surface of the fibroblast, so the procollagen molecule is converted into a tropocollagen molecule, which is further transformed into collagen fibrils. [12-14]. However, type IV collagen does not lose residual peptides after the secretion by fibroblasts. These peptides promote the formation of long fibrils by means of lateral condensation “side by side” [14]. First, the two chains are joined by C-terminal divisions to form dimers that associate with the N-terminals with three other molecules and thus spreading wider. [14]. The result of this association is a mobile multilayer structure stabilized by disulfide and other covalent bonds. [14]. The composition of the basement membrane, in addition to collagen Type IV, also includes heparan sulfate proteoglycan (perlecan) and glycoproteins (laminin and entactin) [14].

Thus, we think that the increase in collagen-IV content in bronchoalveolar fluid in COPD is the evidence of increased fibroblast activity (against the background of microcirculation disorders, activation of lipid peroxidation and hypoxia), and thus manifests the thickening of basement membranes, as well as the violation of both metabolic processes in their own biological systems and the dissociation of a number of dosage forms, especially inhalations. The decrease in collagen-IV content under the influence of treatment with tiotropium bromide is a prominent evidence of physiological course of repair processes, inhibition of neocollagenogenesis in the bronchi and the implementation of a cascade of effects of remodeling inhibition.

CONCLUSIONS

1. Verification of COPD II degree of bronchial obstruction, as compared with apparently healthy individuals, is accompanied by depression of IFN- γ levels (2.29 times, $p < 0.05$) with an increase in collagen-IV level

- (6.15 times, $p < 0.05$) in bronchoalveolar lavage.
- The use of tiotropium bromide as the background therapy of COPD II degree of bronchial obstruction provides a significant reduction in collagen-IV levels in the bronchoalveolar lavage, which depends on the duration of tiotropium use and is the manifestation of antisclerotic activity of this medium.
 - The use of tiotropium bromide as the background therapy of COPD II degree of bronchial obstruction along with the stabilization of clinical and laboratory indices is accompanied by the restoration of IFN- γ levels in the bronchoalveolar lavage, which increases with the prolongation of tiotropium administration, eliminates antigen load and contributes to the physiological sanitation of the inflammatory process in the bronchi.

The prospect for the follow-up studies: is to visualize morphological changes at both cellular and subcellular levels, which would allow us to evaluate the obtained data on the effects of background treatment of COPD.

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ORCID and contributionship:

Mariana Kulynych-Miskiv: 0000-0001-6091-5438^{A,D}

Mykola Ostrovskyy: 0000-0002-3922-0583^{A,F}

Liliia Todoriko: 0000-0002-0117-6513^F

Khrystyna Volnytska: 0000-0002-2488-5582^F

Kseniia Ostrovska: 0000-0002-2402-4539^E

Iryna Savelikhina: 0000-0003-2219-8460^B

Olexander Varunkiv: 0000-0002-9403-9701^B

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mariana Kulynych-Miskiv

Ivano-Frankivsk National Medical University
2 Halytska St., 76018 Ivano-Frankivsk, Ukraine
tel: +38(0342)53 32 95
e-mail: mariana.kulynychmiskiv@gmail.com

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A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

TYPE 2 DIABETES MELLITUS PREVALENCE IN PATIENTS WITH CARDIOVASCULAR DISEASES

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Olena V. Iuzvyshyna, Oksana L. Baranova, Yuliia V. Savitska, Natalia V. Shchepina, Iryna V. Oliinyk, Nina V. Konovalova, Viktor V. Khomovskiy

NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

The aim: To estimate type 2 diabetes mellitus (T2DM) prevalence in patients who came for a consultation to the private practice cardiologist.

Materials and methods: 97 patients with cardiovascular diseases (CVD), visited a cardiologist in private medical center, were included (mean age 49,5 (42,8; 58,3) years, 43 (44.3 %) males) and T2DM prevalence was estimated.

Results: 84 (86.6 %) patients had arterial hypertension, 19 patients (19.6 %) had coronary artery disease. Mean body mass index was 29,4 (25,2; 33,4) kg/m². Waist circumference above recommended was observed in 78.4 %. 30 patients (30.9 %) were overweight, 44.3 % had abdominal obesity. Almost all patients had atherogenic dyslipidemia. Total T2DM prevalence was 19.6 %, including cases of first-diagnosed diabetes (17.5 %).

Conclusions: T2DM and prediabetes prevalence in our sample of patients with CVD was higher than described for the general population. Patients with cardiovascular diseases require careful assessment of diabetes risk factors for its timely detection and for possibly improving the outcomes.

KEY WORDS: type 2 diabetes mellitus, cardiovascular diseases, dyslipidemia, arterial hypertension

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is well known risk factors for the development of cardiovascular diseases (CVD) and cardiovascular mortality; in turn, cardiovascular disease is one of the leading causes of death in diabetes. Meta-analysis of 102 prospective studies showed that diabetes mellitus in general confers a two-fold excess risk of vascular outcomes (coronary heart disease, ischaemic stroke, and vascular deaths), independent of other risk factors, especially in those with long-standing diabetes [1]. Patients with diabetes develop atherosclerosis 7-10 years earlier compared with non-diabetic patients, and can be revealed at the stages of impaired glucose tolerance [2]. Type 2 diabetes mellitus can debut directly as vascular complications - myocardial infarction, cerebral stroke. Even prediabetes is already not only an intermediate stage in the T2DM development, but also an independent risk factor for cardiovascular diseases. The elevated risk of coronary artery disease (CAD) starts at glucose levels below the cut-off point for DM (HR=1.11 already for patients with 5.6-6.1 mmol/L), and increases with increasing glucose levels [1]. So it is strongly recommended to identify patients with a high risk of T2DM as early as possible and actively perform the correction of hyperglycemia, dyslipidemia, arterial hypertension and other risk factors for cardiovascular diseases [3].

The presentation of type 2 diabetes is usually less noticeable compared to type 1 and its start may be completely symptomless. As a result, there is often a long pre-diagnos-

tic period and as many as one-third to one-half of people with type 2 diabetes in the population may be undiagnosed [4]. The causes of T2DM are not completely understood but there is a strong correlation with obesity, increasing age, with ethnicity and family history. Type 2 diabetes is "getting younger" today as a result of an increasing prevalence of obesity. According to the data of International Diabetes Federation (IDF), 40.7% cases of diabetes in Europe are undiagnosed. [4].

THE AIM

To estimate T2DM prevalence in patients who came for a consultation to the private practice cardiologist.

MATERIALS AND METHODS

The results of the examination of patients, who went to a private medical center to a cardiologist were analyzed. 97 consecutive patients were included (mean age 49,5 (42,8; 58,3) years, 43 (44.3 %) males). 37 (38.1%) patients were observed by a family doctor and applied for consultation and correction of therapy, 60 (61.9 %) applied independently, as a private practice does not require prior referral. Arterial hypertension (AH), CAD and disorders of glucose metabolism were assessed according to the current recommendations [1,5,6,7]. All patients signed an informed agreement to use their data, patients who didn't

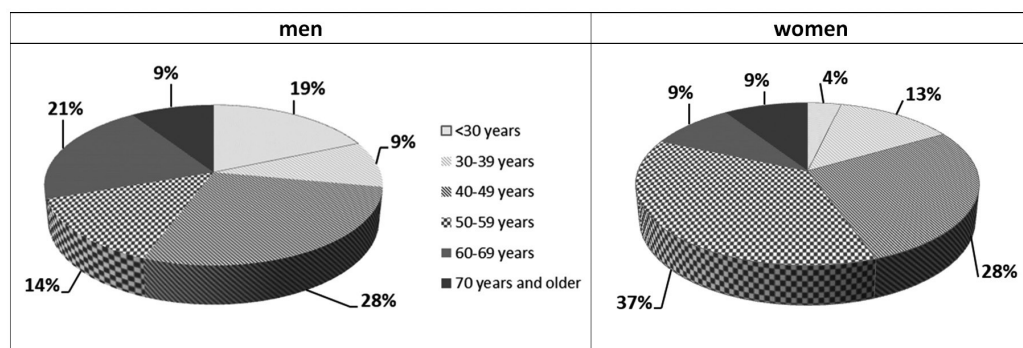


Fig.1. Distribution of the examined patients by age groups.

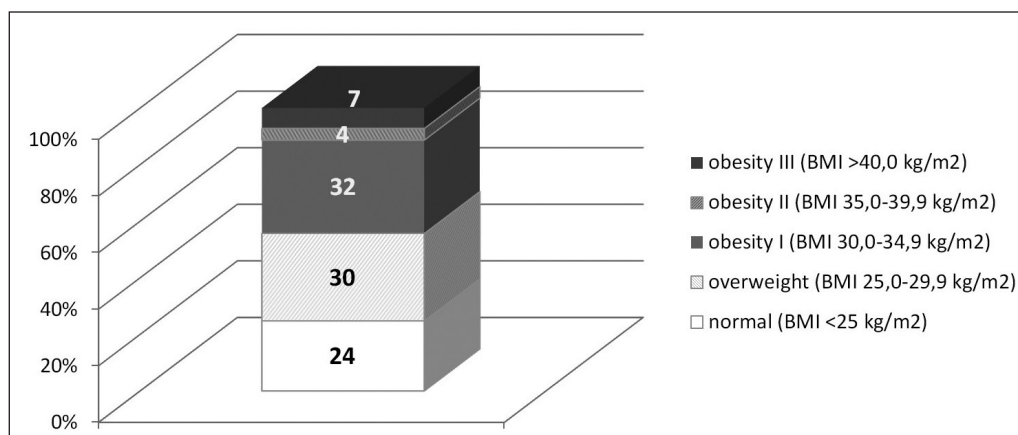


Fig.2. Distribution of the examined patients by BMI groups.

agree was excluded. The statistical analysis was performed using Excel v.10.0 (Microsoft) and STATISTICA v.13.3 (Softserve) programs. The numerical values are reported as median (lower and upper quartiles) and as a proportion of the sample size.

RESULTS

These were mainly young and middle-aged patients (fig.1), which was most likely due to the higher income level of these age categories and, accordingly, the possibility of receiving paid medical services. Mean age was 49,5 (42,8; 58,3) years. Women were slightly older than men (mean age 50.5 (48.0; 57.5) vs 46.5 (40.7; 60.5)), although this was not reliable ($p=0.062$). This may reflect the later manifestation of cardiac pathology in women.

Most of the patients presented with AH (only 5 patients had normal blood pressure (BP), these were young people, the reason for the visit were heart rhythm disturbance - sinus tachycardia and extrasystole). 8 patients (8.2 %) had high normal BP, 84 (86.6 %) had AH (57.2 % of them had grade 1 hypertension, 20.2 and 22.6 % - 2nd and 3d grades respectively). 19 patients (19.6 %) had CAD (5 patients with history of myocardial infarction, 2 patients after percutaneous coronary interventions). 2 patients presented with myocarditis. 22 (22.7 %) patients had heart failure (77.3 and 22.7 % with NYHA FC II and III respectively). 2 patients presented with T2DM. 8.2 % were smokers (all 8 patients were males). It was revealed family history of

cardiac disease in 32 (32.9 %) patients and diabetes family history in 19 (19.6 %) patients.

Thus, most patients in our sample were “non-severe” cardiological patients, many of them were “on the start” of cardiovascular disease, having relatively short duration of AH.

At the same time, mean body mass index (BMI) in our sample was 29,4 (25,2; 33,4) kg/m², so only 24 patients (24.7 %) had normal BMI. Even in the group with normal BMI 3 patients had waist circumference higher than 94 cm for men and 80 cm for women. 30 patients (30.9 %) were overweight (all with waist circumference above the recommended) and all the other (44.3 %) had abdominal obesity (fig.2). Thus, waist circumference above recommended (as one of the components of metabolic syndrome) was observed in 78.4 % of patients. 17 patients had fasting blood glucose (FBG) results at first visit, 8 of them had increased levels (3 patients with FBG > 7.0 mmol/l, 5 patients had FBG from 5.6 to 6.9 mmol/l).

Patients were referred for further examination, including, among other prescription, lipid profile and haemoglobin A1c (HbA1c) and two-hour oral glucose tolerance test for certain patients. Only 40 patients (41.2 %) came for a follow-up visit with the test results. Almost all patients had atherogenic dyslipidemia (total cholesterol level was 6,25 (5,60; 6,42) mmol/l, high-density lipoprotein cholesterol was 1,27 (1,03; 1,60) mmol/l, low-density lipoprotein cholesterol was 3,98 (3,43; 4,32) mmol/l, triglycerides level was 1,71 (1,40; 2,05) mmol/l (only one 19-years old patient

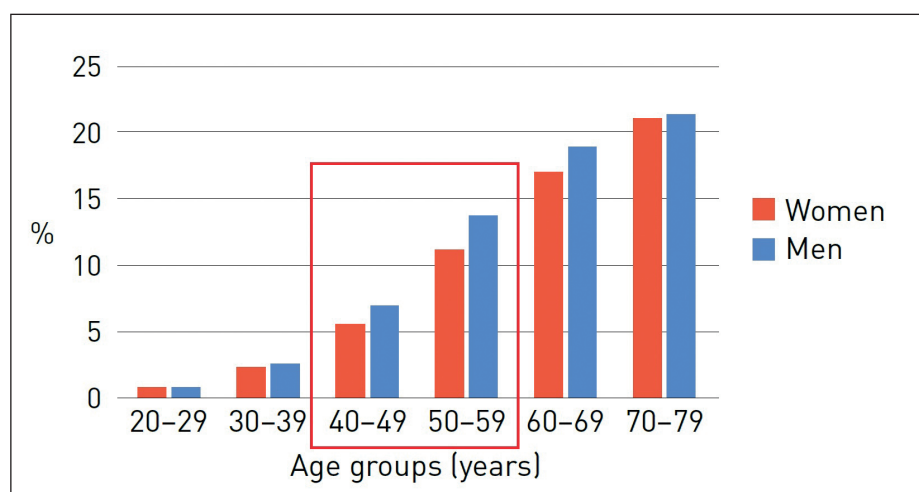


Fig.3. Prevalence (%) estimates of diabetes by age and sex, IDF Europe Region, 2019 (adapted from IDF Diabetes Atlas, 9th edition [4])

had normal lipid profile (that was prescribed considering his family history)).

Haemoglobin A1c level over 6.5 % was revealed in 17 (17.5 %) and that were a cases of first-diagnosed diabetes. Taking into account patients that presented with T2DM on first visit, total T2DM prevalence in our middle-aged sample was 19.6 %. 5 patients (5.2 %) had HbA1c level from 5.9 to 6.4 %, meeting the criteria for prediabetes according to ADA 2019 recommendation. Among patients who did not come for a follow-up visit, 4 patients had FBG from 5.6 to 6.9 mmol/l and 1 patients had FBG > 7.0 mmol/l.

DISCUSSION

Our study was relatively small and thus not entirely representative, but should accent on the need for early diagnosis of diabetes, especially in patients with cardiovascular diseases (CVD), because T2DM and CVD are common comorbidities, mutually aggravating the course of each other. Total T2DM prevalence in our study (19.5 %) was higher than that given in the “Dibetes Atlas 2019” [4] for this age category (fig.3), but, on the one hand, according to the IDF, Europe prevalence of undiagnosed diabetes (20–79 years) was 40.7 % in 2019, and, on the other hand, the prevalence was estimated in patients with CVD, and our patients had high prevalence of abdominal obesity. The results could have been even higher if all patients were completely examined (only a part of patients came for a follow-up visit with the test results). In any case, these results indicate that, if the recommendations are followed (testing for prediabetes and/or type 2 diabetes in asymptomatic people should be considered in adults of any age who are overweight or obese (BMI>25 kg/m² or >23 kg/m² in Asian) and who have one or more additional risk factors for diabetes; for all people, testing should begin at age 45 years [7]), it improves the early diagnosis of diabetes and gives patients a chance to improve their outcomes. It is also very important to reveal cases of prediabetes because this patients have an increased risk of diabetes and already heightened risk of CVD, and their detection “opens the

door to interventions that can lead to the prevention of type 2 diabetes” [4]. In our study patients meeting the criteria for prediabetes were identified (5.2 % of patients with HbA1c level from 5.9 to 6.4 %; and 4 patients had FBG from 5.6 to 6.9 mmol/l on the first visit who did not come for a follow-up visit).

CONCLUSIONS

1. T2DM and prediabetes prevalence in our sample of patients with CVD was higher than described for the general population.
2. Patients with cardiovascular diseases require careful assessment of diabetes risk factors for its timely detection and for possibly improving the outcomes.

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ORCID and contributionship:

Olena V. Iuzvyshyna: 0000-0003-1403-0035 ^{A,F}

Oksana L. Baranova: 0000-0002-4320-3137 ^{A,D}

Yuliia V. Savitska: 0000-0002-9794-4483 ^B

Natalia V. Shchepina: 0000-0001-2345-6789 ^B

Iryna V. Oliinyk: 0000-0001-6034-3580 ^C

Nina V. Konovalova: 0000-0001-5035-5529 ^E

Viktor V. Khomovskyi: 0000-0002-6478-492X ^B

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Oksana L. Baranova

Vinnytsia National Pirogov Memorial Medical University

8/1 Vodoprovizna st., 29000 Khmelnytskyi, Ukraine

tel: +380971325952

e-mail: baranova.oksana@vnmu.edu.ua

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A - Work concept and design, **B** - Data collection and analysis, **C** - Responsibility for statistical analysis,

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

ORIGINAL ARTICLE

EVALUATION OF BACTERIAL CONTAMINATION IN THE INANIMATE ENVIRONMENT SURFACES IN ACUTE CARE HOSPITALS IN KYIV, UKRAINE

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Aidyn G. Salmanov^{1,2,3}, Dmytro V. Shcheglov³, Oleh Svyrydiuk³, Ihor M. Bortnik³, Maryna Mamonova⁴, Anna S. Krylova¹, Maxim S. Gudym³

¹SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

²INSTITUTE OF PEDIATRICS, OBSTETRICS AND GYNECOLOGY OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, KYIV, UKRAINE

³SCIENTIFIC-PRACTICAL CENTER OF ENDOVASCULAR NEURORADIOLOGY OF NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, KYIV, UKRAINE

⁴BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

The aim: To evaluate the quality of cleaning and disinfection of surfaces scheduled for daily cleaning and degree of bacterial contamination of hospital rooms and the patients' inanimate environment in Kyiv acute care hospitals, Ukraine.

Materials and methods: We performed a multicenter prospectively study of the quality of cleaning and disinfection of surfaces scheduled for daily cleaning in 9 acute care hospitals by use of an ultraviolet fluorescence targeting method and microbial methods.

Results: A total 9,104 environmental samples from were collected and tested. The cleaning and disinfection of surfaces were not being performed properly in most cases. Complete removal of the mark was 49.1%, partial removal was 37.5%, and mark was still visible, i.e. this area had not been processed was 13.4% when the ultraviolet fluorescence targeting method procedures were used, respectively. The predominant bacterial agents in hospital environment surfaces were: *Escherichia coli*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus* spp., *Citrobacter* spp., *Acinetobacter* spp., and *Enterococcus* spp. The overall proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriaceae was 31.5% and of methicillin-resistance in *Staphylococcus aureus* (MRSA) 14.9%. Vancomycin resistance was observed in 5.2% of isolated enterococci (VRE). Resistance to third-generation cephalosporins was observed in 12.7% *E.coli* isolates and was in 11.2% *K. pneumoniae* isolates. Carbapenem resistance was identified in 24.7% of *Paeruginosa* isolates and 59.3% of *Acinetobacter* spp. isolates.

Conclusions: In a hospital rooms, patient environmental surfaces can be a vehicle for the transmission of multidrug-resistant (MDR) bacterial agents that cause healthcare-associated infections.

KEY WORDS: healthcare-associated infections; surface contamination, cleaning, disinfection, ultraviolet fluorescence targeting method, antimicrobial resistance

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INTRODUCTION

Healthcare-associated infections (HAIs) are among the most common adverse events in patient care [1]. The emergence and spread of HAIs has become a major public health threat in worldwide. HAIs have been reported to exact a tremendous toll on patients, families and systems of care, resulting in increased morbidity and mortality and increased healthcare costs.

According to literature, HAIs contribute to patient morbidity and mortality with an estimated 1.7 million infections and 99,000 deaths costing USD \$28-34 billion annually in the United States alone [2]. HAIs annually account for 37,000 attributable deaths in Europe. Annual financial losses due to HAIs are also significant, as they are estimated at approximately €7 billion in Europe, including direct costs only and reflecting 16 million extra days of hospital stay [3]. The overall prevalence of HAIs in Ukraine was 11.3%. The most frequently reported HAI

types were surgical site infections (60%), respiratory tract infections (pneumonia and lower respiratory tract, 18.4%), bloodstream infections (10.2%), and urinary tract infections (9.5%). Death during hospitalization was reported in 9.7% of HAI cases [4]. Despite major advances in infection control interventions, HAIs remain a major public health problem and patient safety threat worldwide.[5].

For several decades, environmental surfaces in hospitals were considered to play little or no role in the transmission of HAIs. However, a growing body of evidence suggests that contaminated environmental surfaces can contribute to the transmission of HAIs pathogens [6]. According to the literature, in addition to hand hygiene and reprocessing of medical products, cleaning and disinfection of surfaces is also an important issue in the prevention of germ transmission and by implication infections [7,8]. Accordingly, cleaning and disinfecting environmental surfaces in patient care areas are now recognized as important elements of infection control

programs [9,10]. As a result, there is increasing interest in new technologies that can reliably decontaminate environmental surfaces in healthcare facilities. In recent years, a variety of interventions have been shown to be effective in improving cleaning and disinfection of surfaces.

Currently, in Ukrainian hospitals and other health care settings use more 500 antiseptics and disinfectants for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of HAIs. However, there is little understanding as to if current environmental surface disinfection practices reduce pathogen load, and subsequently HAIs in hospitals. In Ukraine, there are no studies examining how the risk of an transmission of HAI pathogens is associated with bacterial contamination of hospital rooms and the patients' inanimate environment.

THE AIM

To evaluate the quality of cleaning and disinfection of surfaces scheduled for daily cleaning and degree of bacterial contamination of hospital rooms and the patients' inanimate environment in Kyiv acute care hospitals, Ukraine.

MATERIALS AND METHODS

STUDY DESIGN

We performed from January 10st, 2021 to August 31st, 2021 a multicenter prospectively study of the quality of cleaning and disinfection of surfaces scheduled for daily cleaning in 9 acute care hospitals of Kyiv city (Ukraine) by use of an ultraviolet fluorescence targeting method (UVM) and microbial (cultural) methods. All hospitals transferred information on the quality of structure. Process quality

Table I. Monitoring the quality of the cleaning and disinfection of surfaces scheduled for daily cleaning by use of an ultraviolet fluorescence targeting method (UVM) in acute care hospitals in Kyiv, Ukraine (2021)

Hospital	Number of samples	Removal of marking from surfaces with a fluorescent liquid					
		complete removal		partial removal		mark was visible	
		n	%	n	%	n	%
A	1184	645	54.5	368	31.1	171	14.4
B	1120	618	55.2	385	34.4	117	10.4
C	1040	529	50.9	362	34.8	149	14.3
D	960	504	52.5	344	35.8	112	11.7
E	1040	481	46.3	422	40.6	137	13.2
F	1056	484	45.8	434	41.1	138	13.1
G	880	428	48.6	345	39.2	107	12.2
H	912	404	44.3	381	41.8	127	13.9
I	912	377	41.3	375	41.1	160	17.5
Total	9104	4470	49.1	3416	37.5	1218	13.4

Table II. Trend the quality of cleaning and disinfection of surfaces using ultraviolet fluorescence (UVM) targeting in acute care hospitals in Kiev, Ukraine (2021)

Hospital	Number of samples	Removal of marking from surfaces with a fluorescent liquid											
		Mondays			Wednesdays			Saturdays			Sundays		
		complete removal %	partial removal %	mark was visible %	complete removal %	partial removal %	mark was visible %	complete removal %	partial removal %	mark was visible %	complete removal %	partial removal %	mark was visible %
A	1184	67.9	27.0	5.1	64.9	30.1	5.1	55.4	34.5	10.1	29.7	32.8	37.5
B	1120	67.1	28.9	3.9	65.4	31.8	2.9	53.2	42.9	3.9	35.0	33.9	31.1
C	1040	59.6	27.7	12.7	55.8	36.9	7.3	45.0	48.1	6.9	43.1	26.5	30.4
D	960	62.1	29.2	8.8	62.9	35.4	1.7	54.6	40.8	4.6	30.4	37.9	31.7
E	1040	52.7	36.9	10.4	54.2	41.5	4.2	50.8	42.3	6.9	27.3	41.5	31.2
F	1056	47.7	40.9	11.4	48.5	48.5	3.0	45.1	48.9	6.1	42.0	26.1	31.8
G	880	46.4	44.1	9.5	60.5	37.7	1.8	55.5	40.9	3.6	32.3	34.1	33.6
H	912	45.2	45.2	9.6	51.3	46.1	2.6	50.9	44.3	4.8	29.8	31.6	38.6
I	912	42.1	43.0	14.9	47.8	49.1	3.1	43.0	51.8	5.3	32.5	20.6	46.9
Total	9104	55.2	35.4	9.4	57.1	39.3	3.6	50.4	43.6	5.9	33.7	31.8	34.6

Table III. Monitoring the effect of the cleaning and disinfection of surfaces on different department/wards by ultraviolet fluorescence (UVM) targeting in acute care hospitals in Kiev, Ukraine (2021)

Department/wards	Number of samples	Removal of marking from surfaces with a fluorescent liquid					
		complete removal		partial removal		mark was visible	
		n	%	n	%	n	%
General surgery	680	388	57.1	238	35.0	54	7.9
Digestive tract surgery	320	148	46.3	96	30.0	76	23.8
Cardiovascular surgery	360	210	58.3	138	38.3	12	3.3
Ear/nose/throat surgery	480	240	50.0	170	35.4	70	14.6
Orthopedic surgery	560	298	53.2	176	31.4	86	15.4
Neurosurgery	680	360	52.9	266	39.1	54	7.9
Urology	660	345	52.3	221	33.5	94	14.2
Burns care	460	300	65.2	104	22.6	56	12.2
Haematology	410	233	56.8	157	38.3	20	4.9
Pneumology	470	240	51.1	180	38.3	50	10.6
Obstetrics/maternity	680	360	52.9	264	38.8	56	8.2
Gynaecology	680	320	47.1	292	42.9	68	10.0
Paediatrics general	680	280	41.2	304	44.7	86	12.6
Medical ICU	420	140	33.3	189	45.0	91	21.7
Surgical ICU	420	157	37.4	159	37.9	104	37.9
Paediatric ICU	420	170	40.5	182	43.3	68	43.3
Neonatal ICU	364	141	38.7	125	34.3	98	34.3
Mixed and other ICU	360	140	38.9	145	40.3	75	40.3
Total	9104	4470	49.1	3416	37.5	1218	13.4

was obtained through direct observation during cleaning and disinfection of rooms and their plumbing units. All participating hospitals were required to have a clinical microbiology laboratory with the capacity to process cultures and at least one intensive care unit (ICU). The study was conducted in hospital wards, operating theatres and intensive care units.

DATA COLLECTION

In our study, as part of a questionnaire-based survey, data was obtained regarding the staff training and quality control, the interface of the responsibilities of house cleaning and nursing personnel, the work instructions (standard operating procedures (SOP)), the cleaning performance on weekends and holidays.

We took samples on Mondays, Wednesdays, Saturdays, and Sundays for one month and took samples from near-and extended patient areas. Infection control practitioners of the respective hospitals had marked definite points in fluorescent ink, according to the CDC recommendation [11]. On the day of the control visit, reprocessing of at least 5 four-bed rooms and bathrooms was monitored in every hospital. In during the control visit infection control

practitioners, if and how these points had been removed by cleaning was determined using an ultraviolet flashlight. Cleaning performance was measured by complete removal of UVM, i.e. marking surfaces with a fluorescent liquid and testing if this mark has been sufficiently removed by cleaning and removed of bacterial contamination of hospital rooms and the patients' inanimate environmental surfaces. Complete removal of the mark was scored as two points, partial removal was given one point, and zero points were awarded if the mark was still visible, i.e. this area had not been processed.

MICROBIAL METHODS

Microbiological samples were taken from the surfaces of near-and extended patient areas. In each hospital, two infection control practitioners carried out the sampling. To sample a large surface, we used RODAC plate, 55 mm in diameter. A RODAC plate, 55 mm in diameter, was pressed on the surface to be tested, and then incubated at 36°C for 48 h. Microbial isolates were identified using standard microbiological techniques. Antibiotic susceptibility testing was performed by using the disk diffusion method according to the recommendations of the European Committee on Antimicrobial Susceptibility

Table IV. Monitoring the effect of the cleaning and disinfection of surfaces on different items in hospital room by ultraviolet fluorescence (UVM) targeting in acute care hospitals in Kiev, Ukraine (2021)

Environmental items in hospital room	Number of samples	Removal of marking from surfaces with a fluorescent liquid					
		complete removal		partial removal		mark was visible	
		n	%	n	%	n	%
Bed rails	560	270	48.2	261	46.6	29	5.2
Tray table	610	378	62.0	208	34.1	24	3.9
Bedside table handle	470	216	46.0	237	50.4	17	3.6
Bedside table	610	297	48.7	301	49.3	12	2.0
Chair	510	430	84.3	76	14.9	4	0.8
Room sink	430	192	44.7	224	52.1	14	3.3
Room light switch	640	173	27.0	421	65.8	46	7.2
Room inner door knob	640	176	27.5	441	68.9	23	3.6
Door handle	620	170	27.4	413	66.6	37	6.0
IV pump control	180	139	77.2	37	20.6	4	2.2
Multi-module monitor controls	460	137	29.8	167	36.3	156	33.9
Multi-module monitor touch screen	460	132	28.7	107	23.3	221	48.0
Multi-module monitor cables	614	121	19.7	207	33.7	286	46.6
Ventilator control panel	160	71	44.4	75	46.9	14	8.8
Infusion pump	140	68	48.6	65	46.4	7	5.0
Switches of intravenous pumps	140	51	36.4	71	50.7	18	12.9
Bathroom door	180	178	98.9	2	1.1	0	0
Shower	180	173	96.1	7	3.9	0	0
Basin fitting	180	169	93.9	11	6.1	0	0
Shower fitting	180	172	95.6	8	4.4	0	0
Bathroom sink	180	171	95.0	9	5.0	0	0
Bathroom light switch	180	171	95.0	9	5.0	0	0
Bathroom inner door knob	180	168	93.3	0	0	12	6
Bathroom handrails by toilet	200	91	45.5	11	5.5	98	49.0
Toilet seat	200	89	44.5	27	13.5	84	42.0
Toilet flush handle	200	67	33.5	21	10.5	112	56.0
Total	9104	4470	49.1	3416	37.5	1218	13.4

Testing (EUCAST). In our study, strains in the intermediate range were classified as resistant for data analysis.

ETHICS

The Shupyk National Healthcare University of Ukraine Ethics Committee approved this study.

STATISTICAL ANALYSIS

Descriptive statistical analysis was performed to provide median, minimum-maximum values range, and mean standard deviation. Parametric or nonparametric tests were applied on the basis of data distribution. The Wilcoxon, Mann-Whitney, and Fisher's Exact tests were run to analyze data statistically. For the statistical analysis, we used a significance level of $p < 0.05$.

RESULTS

EVALUATION BY ULTRAVIOLET FLUORESCENCE TARGETING METHOD

A total 9,104 environmental samples from were collected and tested for the evaluations of the quality of cleaning and disinfection of surfaces scheduled for daily cleaning in 9 acute care hospitals by use of an ultraviolet fluorescence targeting method (UVM). Cleaning and disinfection of surfaces were not being performed properly in most cases. Complete removal of the mark was 49.1% (4470/9104) [95% CI 48.4%, 49.9%, $p < 0.0001$], partial removal was 37.5% (3416/9104) [95% CI 36.7%, 38.3%, $p < 0.0001$], and mark was still visible, i.e. this area had not been processed was 13.4% (1218/9104) [95% CI 12.4%, 14.4%, $p < 0.0001$] when the UVM procedures were used, respectively.

Table V. Distribution of microorganisms isolated from the patients' inanimate environment surfaces in acute care hospitals in Kyiv, Ukraine (2021)

Microorganisms	All isolates (n=11723)	Percentages, %
<i>Gram-positive cocci</i>	1672	14.3
<i>Staphylococcus aureus</i>	248	2.1
<i>Coagulase-negative staphylococci</i>	529	4.5
<i>Enterococcus spp.</i>	717	6.1
<i>Streptococcus spp.</i>	178	1.5
<i>Gram-negative bacilli</i>	10051	85.7
<i>Escherichia coli</i>	3374	28.8
<i>Citrobacter spp.</i>	822	7.0
<i>Enterobacter spp.</i>	1401	12.0
<i>Klebsiella pneumoniae</i>	991	8.5
<i>Proteus spp.</i>	892	7.6
<i>Serratia spp.</i>	341	2.9
<i>Acinetobacter spp.</i>	788	6.7
<i>Pseudomonas aeruginosa</i>	1374	11.7
<i>Stenotrophomonas maltophilia</i>	68	0.6
Total	11723	100.0

Cleaning performance varied significantly between the 9 acute care hospitals. Results of monitoring the quality of the cleaning and disinfection of surfaces scheduled for daily cleaning by use of an ultraviolet fluorescence targeting method (UVM) in acute care hospitals are presented in Table I.

There were significant differences in the quality of cleaning and disinfecting environmental surfaces in hospitals on Mondays, Wednesdays, Saturdays, and Sundays. The best results of cleaning the patients' inanimate environment surfaces were achieved in Mondays and Wednesdays, the worst results in Saturdays, and Sundays (Table II). The best results of were achieved in Burns care, Cardiovascular surgery, General surgery, and Haematology departments, the worst results in intensive care units. Results of monitoring the effect of the cleaning and disinfection of surfaces on different department/wards by ultraviolet fluorescence (UVM) targeting in acute care hospitals are presented in Table III.

Evaluation the priority sites most frequently contaminated and touched by patients and/or healthcare workers found significant differences in the effectiveness of cleaning and disinfecting surfaces on various items in the hospital wards. The results of monitoring the effect of the cleaning and disinfection of surfaces on different items in hospital room by ultraviolet fluorescence (UVM) targeting in acute care hospitals are presented in Table IV.

EVALUATION BY MICROBIOLOGICAL METHOD

A total of 11723 strains isolated from 9104 the patients' inanimate environment surfaces. Gram-positive organisms accounted for 14.3% (1672/11723) [95% CI 14.4%, 15.2%, $p < 0.0001$] of all strains and gram-negative or-

ganisms accounted 85.7% (10051/11723) [95% CI 85.4%, 86.1%, $p < 0.0001$], respectively. Enterobacteriaceae were the most frequently isolated group of organisms from the patients' inanimate environment surfaces (67.6%, 95% CI 67.1%, 68.1%, $p < 0.0001$). The predominant bacterial agents were: *E. coli* (28.8%), *Enterobacter spp.* (12%), *P. aeruginosa* (11.7%), *K.pneumoniae* (8.5%), *Proteus spp.* (7.6%), *Citrobacter spp.* (7%), *Acinetobacter spp.* (6.7%), and *Enterococcus spp.* (6.1%), followed by Coagulase-negative staphylococci (4.5%), *Serratia spp.* (2.9%), *S. aureus* (2.1%), *Streptococcus spp.* (1.5%), and *Stenotrophomonas maltophilia* (0.6%) (Table V). Evaluation the priority sites most frequently contaminated and touched by patients and/or healthcare workers found significant differences degree of bacterial agents contamination of environmental items in hospital rooms and the patients' inanimate environment (Table VI).

Because most commensally bacteria have natural gene transfer mechanisms and can be resistant to multiple antimicrobials, it is important to characterize the strains that have been isolated from environmental surfaces. Antimicrobial susceptibility tests were performed on a total of 1672 isolates of Gram-positive cocci and 10051 gram-negative organisms. The antimicrobials used in antimicrobial susceptibility testing included those commonly used as therapeutic agents in Ukraine. Varying degrees of resistance to most antimicrobials tested were found. Staphylococcal isolates showed susceptibility to most antimicrobials tested, although there were some differences depending on the environmental surfaces. No strains resistant to linezolid, teicoplanin, vancomycin, tigecycline, and fusidic acid were found. Methicillin-resistance was observed in 14.9% of *S. aureus*.

Table VI. Distribution of microorganisms isolated from the priority sites most frequently contaminated and touched by patients and/or healthcare workers in acute care hospitals in Kiev, Ukraine (2021)

Environmental items in hospital room	Microorganisms
Bed rails	<i>E.coli</i> , <i>Enterococcus</i> spp., <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Proteus</i> spp.
Tray table	<i>S.aureus</i> , <i>Enterococcus</i> spp., <i>CNS</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp., <i>E.coli</i> ,
Bedside table handle	<i>S.aureus</i> , <i>Enterococcus</i> spp., <i>CNS</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp., <i>E.coli</i> ,
Bedside table	<i>S.aureus</i> , <i>Enterococcus</i> spp., <i>CNS</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp., <i>E.coli</i> ,
Chair	<i>Enterobacter</i> spp., <i>Citrobacter</i> spp., <i>Proteus</i> spp., <i>CNS</i> , <i>E.coli</i> ,
Room sink	<i>Enterobacter</i> spp., <i>E.coli</i> , <i>Citrobacter</i> spp., <i>P.aeruginosa</i> , <i>Enterococcus</i> spp.,
Room light switch	<i>S.aureus</i> , <i>Enterococcus</i> spp., <i>Streptococcus</i> spp. <i>E.coli</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>S. maltophilia</i>
Room inner door knob	<i>Enterococcus</i> spp., <i>E.coli</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Proteus</i> spp., <i>S.aureus</i> , <i>CNS</i> , <i>Streptococcus</i> spp.
Door handle	<i>Enterococcus</i> spp., <i>E.coli</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Proteus</i> spp., <i>S. maltophilia</i> , <i>S.aureus</i> , <i>Streptococcus</i> spp.
IV pump control	<i>Enterococcus</i> spp., <i>E.coli</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Acinetobacter</i> spp., <i>K. pneumoniae</i> , <i>CNS</i> , <i>Streptococcus</i> spp.
Multi-module monitor controls	<i>Enterococcus</i> spp., <i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Acinetobacter</i> spp., <i>K. pneumoniae</i> , <i>CNS</i> ,
Multi-module monitor touch screen	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Acinetobacter</i> spp., <i>K. pneumoniae</i> , <i>CNS</i> . <i>S. maltophilia</i> , <i>Streptococcus</i> spp.
Multi-module monitor cables	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Acinetobacter</i> spp., <i>K. pneumoniae</i> , <i>S. maltophilia</i> , <i>Proteus</i> spp.,
Ventilator control panel	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>K. pneumoniae</i> , <i>Acinetobacter</i> spp.
Infusion pump	<i>E.coli</i> , <i>Enterobacter</i> spp.
Bathroom door	<i>E.coli</i> , <i>Citrobacter</i> spp., <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i>
Shower	<i>E.coli</i> , <i>Serratia</i> spp. <i>P.aeruginosa</i>
Basin fitting	<i>E.coli</i> , <i>Serratia</i> spp.
Shower fitting	<i>E.coli</i> , <i>Enterococcus</i> spp., <i>Citrobacter</i> spp.,
Bathroom sink	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Proteus</i> spp., <i>S. maltophilia</i>
Bathroom light switch	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Proteus</i> spp., <i>S. maltophilia</i> ,
Bathroom inner door knob	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i>
Toilet seat	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Proteus</i> spp.,
Toilet flush handle	<i>E.coli</i> , <i>Enterobacter</i> spp., <i>Serratia</i> spp. <i>P.aeruginosa</i> , <i>Proteus</i> spp.

Regarding the genus *Enterococcus*, *E. faecalis* isolates and *E. faecium* were not sensitive to those antibiotics to which they are intrinsically resistant (cefuroxime, clindamycin, and trimethoprim-sulfamethoxazole) and 85.3% of them were resistant to erythromycin. Approximately, 20% of the *E. faecalis* isolates displayed resistance to high levels of aminoglycosides (gentamycin, tobramycin) and around 9.1% was resistant to quinolones (ciprofloxacin and levofloxacin). Vancomycin resistance was observed in 5.2% of isolated enterococci (VRE). The overall proportion of extended spectrum beta-lactamases (ESBL) production among Enterobacteriaceae was 31.5%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (36.1%, vs 16.3%, $p < 0.001$). Resistance to third-generation cephalosporins was observed in 12.7% *E.coli* isolates. No strains of *E.coli* resistant to ertapenem were found. Resistance to third-gener-

ation cephalosporins was observed in 11.2% *K. pneumoniae* isolates. Carbapenem resistance was identified in 24.7% of *P.aeruginosa* isolates and 59.3% of *Acinetobacter* spp. isolates.

DISCUSSION

This is the first study in Ukraine were to evaluate the quality of cleaning and disinfection of surfaces scheduled for daily cleaning and degree of bacterial contamination of hospital rooms and the patients' inanimate environment by use of an ultraviolet fluorescence targeting method (UVM) and microbial methods. In this study the cleaning and disinfection of surfaces were not being performed properly in most cases. Complete removal of the mark was 49.1%, partial removal was 37.5%, and mark was still visible, i.e. this area had not been processed was 13.4% when the UVM procedures were

used, respectively. Cleaning performance varied significantly between the 9 acute care hospitals. Contamination of hospital rooms and the patients' inanimate environment surfaces by the bacterial pathogens investigated was found to be frequent and widespread occurrence. The predominant bacterial agents were: *E. coli*, *Enterobacter* spp., *P. aeruginosa*, *K. pneumoniae*, *Proteus* spp., *Citrobacter* spp., *Acinetobacter* spp., and *Enterococcus* spp., followed by Coagulase-negative staphylococci (CNS), *Serratia* spp., *S. aureus*, *Streptococcus* spp., and *Stenotrophomonas maltophilia*.

The increasing emergence and spread of multi-resistant bacteria in hospitals is of great concern and continues to challenge infection control and hospital epidemiology practice worldwide [12]. However, only limited data concerning the colonization of a patient with multi-resistant Gram-positive and Gram-negative strains and the subsequent spread of these strains into the hospital environment are currently available. In our study a significant part of the bacterial agents isolated from the environment surfaces were resistant to many antibiotics. The overall proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriaceae was 31.5% and of methicillin-resistance in *S. aureus* (MRSA) 14.9%. Vancomycin resistance was observed in 5.2% of isolated enterococci (VRE). Resistance to third-generation cephalosporins was observed in 12.7% *E. coli* isolates and was in 11.2% *K. pneumoniae* isolates. Carbapenem resistance was identified in 24.7% of *P. aeruginosa* isolates and 59.3% of *Acinetobacter* spp. isolates.

According to the literature, micro-organisms in the patients' inanimate environment surfaces scheduled for daily cleaning contribute to HAI [2, 12-16]. Although there is no direct proof, there is mounting evidence that the environment of patients colonized with Gram-positive and Gram-negative bacteria serves as a potential reservoir for cross-transmission and hence, possible nosocomial infections. Patients hospitalized in rooms previously occupied by people infected with HAIs are at increased odds of HAI acquisition compared to patients whose prior room occupant was negative for HAIs.

The environmental transmission pathways of pathogens and HAIs are varied. Measures to reduce the environment as a transmission pathway for HAIs are also varied. Improved cleaning procedures, training environmental service personnel, hand hygiene, and bundled disinfection interventions reduce the concentrations of pathogens on environmental surfaces and reduce HAIs in healthcare facilities. The literature has focused on multimodal strategies in infection prevention and control. Understanding the efficacy of the individual components of multi-modal strategies may help guide bundle development and may aid in decision-making in low-resource settings. However, there has not been a rigorous systematic review of the efficacy of disinfection interventions in situ.

CONCLUSIONS

Our studies have shown that in hospital rooms, most patient environmental surfaces are contaminated with multidrug-resis-

tant bacterial agents and can be a vehicle for the transmission of healthcare-associated infections pathogens. Cleaning and disinfection processes must be improved so that there is a reduction in environmental contamination of frequent-contact surfaces in hospitals. Transmission of infectious agents from contaminated surfaces to a patient may occur via direct contact, indirectly via the hands or gloves of healthcare personnel. Failure to properly disinfect carries risk for person-to-person transmission and transmission of environmental pathogens. To reduce transmission risk of infectious agents from contaminated surfaces to the patient, more attention should be paid to the evidence-based recommendations on the preferred methods for Handwashing, cleaning and disinfecting the healthcare environment. It is important for healthcare personnel to recognize the role of patient healthcare environment as a transmission risk of multidrug-resistant infectious agents and adhere to prevention strategies for healthcare-associated infections based on current international guidelines and the literature. Further studies are needed to confirm our data and elucidate the relative importance of the patient-care items can serve as a source or reservoir for multidrug resistant bacteria in hospitals, including causation between contamination of a pathogen with a fomite and actual HAIs, elucidation of direct and indirect transmission mechanisms via a patient-care items using advanced molecular typing, and improvement of adherence to cleaning and disinfection practice.

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ORCID and contributionship:

Aidyn G. Salmanov: 0000-0002-4673-1154 ^{A, C, F}
Dmytro V. Shcheklov: 0000-0003-1465-8738 ^{B, E, F}
Oleh Svyrydiuk: 0000-0001-7455-0396 ^{B, C, F}
Ihor M. Bortnik: 0000-0001-8072-6570 ^{B, C, F}
Maryna Mamonova: 0000-0002-0697-4864 ^{B, C, F}
Anna S. Krylova: 0000-0002-7953-187X ^{B, C, F}
Maxim S. Gudym: 0000-0001-8224-8314 ^{B, C, F}

Conflict of interest:

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Aidyn G. Salmanov

Shupyk National Healthcare University of Ukraine,
9 Dorohozhytska St., 04112, Kyiv, Ukraine
tel: +380667997631
e-mail: mozsago@gmail.com

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ORIGINAL ARTICLE

ANTIBODIES TO MICROBIAL ANTIGENS AND CYTOKINES IN THE CELLS OF THE PALATINE TONSILS AND SERUM OF CHILDREN WITH PALATINE TONSILS HYPERTROPHY AND CHRONIC TONSILLITIS

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Oleksandr Bredun, Ilona Kosakivska

SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

ABSTRACT

The aim: The aim of the study is to compare the class G antibody content in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis to: streptolysin-O of *Str. haemolyticus*, protein-A of *S. aureus*, proteoglycans of *Klebsiella spp.*, as well as to compare the content of interleukins 1 β , 10, TNF- α , γ -IFN and lactoferrin in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis.

Materials and methods: We studied tonsils of 33 children aged 4-18 years with hypertrophy of palatine tonsils (HPT) and with chronic tonsillitis (CT). The content of interleukins 1 β , 10, TNF- α , γ -IFN and lactoferrin in tonsil lysate and serum was determined by immunofluorescence assay. Antistreptolysin O was studied by neutralization test of micromethod; class G antibodies to protein A of *S. aureus* and proteoglycans of *Klebsiella spp.* were studied by treponema pallidum hemagglutination assay. All the results were statistically processed using U-test (Mann-Whitney-Wilcoxon test) and Fisher's z-transformation.

Results: The serum and tissue lysate from tonsils of patients with HPT showed significantly high level of antibodies to streptolysin O in comparison with similar studies of substrates from patients with CT. Anti-inflammatory cytokine IL-10 was detected only in the serum of patients with CT. The TNF- α concentration in the lysates of tonsils in the group of patients with HPT was 2 times higher than in the group of patients with CT. The γ -IFN concentration was significantly lower both in the serum and in the lysates of tonsils of patients with CT. The content of lactoferrin in the lysates of patients with CT was 3 times higher ($P < 0.05$) than in the lysates of patients with HPT.

Conclusions: The results indicate a significant difference in the state of antibodies to microbial antigens and cytokines production in case of HPT and CT. In tonsils with HPT, there predominate reactions of antibody production to bacterial antigens and antiviral reactions like a high-level cytokines TNF- α and γ -IFN in tissue lysate of palatine tonsils.

KEY WORDS: Hypertrophy of palatine tonsils, chronic tonsillitis, immunity factors, interleukins, lactoferrin, antibodies

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INTRODUCTION

A very common abnormality in the clinical status of palatine tonsils is the state of their hypertrophy at an early age. Due to the clinical need, such tonsils are often the object for clinical intervention which involves partial resection of tonsillar tissue, that is tonsillotomy (partial tonsillectomy). The state of the palatine tonsil after such an intervention can hardly be defined as functionally preserved. In addition, taking into account present data on the role of this organ in the immune system, the development of the following local immune deficiency of mucous membranes can be assumed, an indicator of which is sIgA [1, 2]. The main pathological abnormalities in the state of tonsils are most often manifested as their hypertrophy or chronic inflammation.

Pathological characteristics and immunology research in recent decades have not identified significant differences between these conditions. And the development of new directions in immunology offered real possibilities for a more objective assessment of the tonsillar tissue state, the influence on the systemic and local immune response of various peptides formed in the palatine tonsils in case of hypertrophy and chronic inflammation [3, 4, 5, 6].

THE AIM

The aim of the study is to compare the antibody content in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis to: streptolysin-O of *Str. haemolyticus*, protein-A of *S. aureus*, proteoglycans of *Klebsiella spp.*, as well as to compare the content of interleukins 1 β , 10, TNF- α , γ -IFN and lactoferrin in serum and tissue lysate from tonsils of children with hypertrophy and chronic tonsillitis.

MATERIALS AND METHODS

The content of cytokines and protective proteins in lysates of tonsil cells and serum of children with grade 2-3 hypertrophy according to Brodsky's classification and in those with chronic tonsillitis was studied. The study involved 15 patients with CT and 18 patients with HPT aged 4-18 years.

After surgical removal, tonsillar extracts were immersed in 199 medium containing gentamicin (100 μ g/ml), kept at 40°C, mechanically homogenized and filtered through a nylon sieve; the cell concentration was calculated, adjusted to a standard

Table I. The content of class G antibodies to microbial antigens in the lysates of the cells of tonsils and serum of children with CT and HPT.

Group of patients	Lg2 antibody titers (average) to microbial antigens					
	streptolysin O of <i>Str. haemolyticus</i>		protein A of <i>S. aureus</i>		proteoglycan of <i>Klebsiela spp.</i>	
	Lysate	Serum	Lysate	Serum	Lysate	Serum
CT (n=15)	1.3	4.5	0	4.0	0	2.1
HPT (n=18)	3.5*	8.5*	0	3.25	1.2*	3.6

Note: *P<0.05

Table II. The content of cytokines in the lysates of the cells of tonsils and serum of children with CT and HPT.

Group of patients	Concentration of cytokines, pg/ml							
	IL-1β		IL-10		TNF-α		γ-IFN	
	Lysate	Serum	Lysate	Serum	Lysate	Serum	Lysate	Serum
CT (n=15)	46.0	0.2	0	2.1	130.4	34.2	11.2	8.2
HPT (n=18)	41.6	0.2	0	0	236.0*	29.3	36.5*	19.6*

Note: *P<0.05

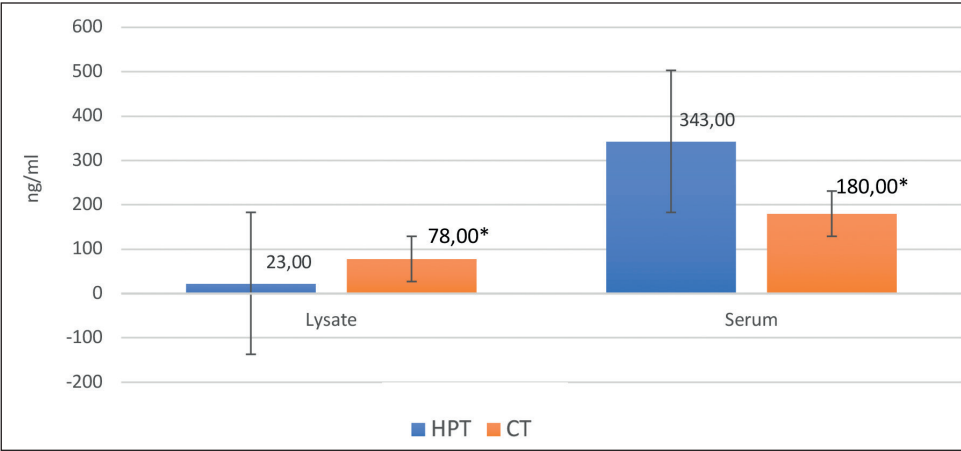


Fig. 1. The content of lactoferrin in the lysates and serum of patients with HPT (n=18) and CT (n=15). *(P<0.05).

content of 1 ml and processed in lysis buffer, followed by incubation in isotonic saline solution and centrifugation.

Supernatants were frozen and stored for 1 month at a temperature of minus (-) 20°C, after which the concentration of cytokines and other peptides and proteins was determined. Serum was obtained from venous blood the day before surgery.

The content of IL-1, IL-10, tumour necrosis factor (TNF-α), interferon gamma (γ-IFN) and lactoferrin in tonsil lysate and serum was determined by immunoenzyme method and assay kits manufactured by “Proteinovy Kontur”, “Cytokin” and “Vector-Best” (RF). A Stat-Fax-2100 reader (USA) was used as an analyzer. Antistreptolysin O was studied by neutralization test of micromethod; antibodies to protein A of *S. aureus* and proteoglycans of *Klebsiela spp.* were studied by treponema pallidum hemagglutination assay using LATEST reagent kits (RF). All the results were statistically processed using U-test (Mann–Whitney–Wilcoxon test) and Fisher’s z-transformation.

RESULTS

The data on the content of class G antibodies to microbial antigens in the lysates of the cells of tonsils and serum of children with CT and HPT are presented in Table I.

As can be seen from Table I, a high level of antibodies is determined in relation to streptolysin O in the serum and lysates of the cells of tonsils in patients with HPT in comparison with similar studies of substrates from patients with CT. The antibodies to protein A of *Staphylococcus aureus* and proteoglycan of *Klebsiela spp.* were almost not detected in the lysates of tonsillar cells, and in the serum, they were at a lower level than to streptolysin O, and did not differ statistically in both comparison groups.

The data on the content of cytokines in the lysates of the cells of tonsils and serum of children with CT and HPT are presented in Table II.

As can be seen from Table II, the concentration of IL-1β in the serum of patients of both groups was minimal (<0.2 pg/ml), and in the lysates of the comparison groups it did not differ statistically. The anti-inflammatory cytokine IL-10 was detected only in the serum of patients with CT. The TNF-α concentration in the lysates of the cells of tonsils in the group of patients with HPT was 2 times higher than in the group of patients with CT; in the serum there was no significant difference between the groups. The γ-IFN concentration was significantly lower both in the serum and lysates of the cells of tonsils of patients with CT.

When studying the concentration of lactoferrin in the lysates and serum of patients with CT and HPT, the following results were obtained (Figure 1).

As can be seen from Figure 1, the content of this iron-containing prodefensin in the lysates of the cells of patients with CT was 3 times higher ($P<0.05$) than in the lysates of patients with HPT, whereas in the serum the opposite significant dependence was determined.

DISCUSSION

The findings indicate a pronounced immunological activity of the tissue of the palatine tonsils both in case of hypertrophy and chronic inflammation. At the same time, a more intense production of antibodies in the tonsils with hypertrophy indicates a greater immunologic capacity of hypertrophied lymphoid tissue. This can also be evidenced by a pronounced inflammatory reaction in the palatine tonsils of patients with HPT, which is confirmed by the level of IL-1 β no less than in the tonsils of patients with CT.

The analysis of the data on the TNF- α content, which was within the normal range in the serum of patients of both groups and significantly higher in the lysates of tonsillar cells of patients with HPT than those with CT, indicates an inflammatory reaction in the hypertrophied palatine tonsils, but mainly to viral antigens. This is also evidenced by a reliably high level of γ -IFN both in the lysates and serum of patients with HPT compared to similar substrates of patients with CT.

The findings indicate that the processes of antibody production to microbial antigens, especially hemolytic streptococcus, have a more intense course in the tonsils in case of HPT than in case of CT. The TNF- α content in hypertrophied tonsils is at a higher level than in CT, which, together with an increased content of γ -IFN, indicates antibody response of the immunity to infectious agents.

In addition, to gain the understanding of the role of individual parts of the immune system and allergy in the immunopathogenesis of both HPT and CT, it is necessary to conduct further studies on the range of abnormalities in the content and functional activity of molecular factors that are important in the implementation of protective reactions in the tonsils in both pathological conditions.

CONCLUSIONS

1. The level of class G antibodies to *Str. haemolyticus* in the lysates of patients with HPT was 2.7 times higher ($P<0.05$) than in those with CT, and in the serum of patients with HPT it was 1.9 times higher ($P<0.05$) than in those with CT.
2. The level of class G antibodies to *Klebsiella spp.* in the serum of patients with HPT was 1.7 times higher ($P<0.05$) than in those with CT.
3. The TNF- α concentration in the lysates of patients with HPT was 1.8 times higher ($P<0.05$) than in those with CT.
4. The γ -IFN concentration was reliably higher ($P<0.05$) in patients with HPT than in those with CT (3.2 times higher in the lysates, and 2.4 times higher in the serum).

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ORCID and contributionship:

Oleksandr Bredun: 0000-0002-5013-7318^{A,F}

Ilona Kosakovska: 0000-0002-6358-2533^{C,D}

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Oleksandr Bredun

Shupyk National Healthcare University of Ukraine
9 Dorohozhytska Str., Kyiv, Ukraine, 04112.
tel: +380505605852
e-mail: alexbredun@gmail.com

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ORIGINAL ARTICLE

COMPARATIVE ANALYSIS OF CLINICAL AND LABORATORY CHARACTERISTICS OF MEASLES IN VACCINATED AND UNVACCINATED CHILDREN IN THE POLTAVA REGION (UKRAINE)

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Valentyna I. Ilchenko, Liudmyla M. Syzova, Kateryna V. Pikul, Iryna L. Dvornyk, Oksana V. Muravlova
POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

The aim: To study the clinical and laboratory characteristics of measles, as well as to compare the course of this disease in vaccinated and unvaccinated children in the Poltava region (Ukraine).

Materials and methods: 104 inpatients with measles were examined: vaccinated (n=27) and unvaccinated (n=77). The patient examination program included: assessment of complaints and anamnestic data obtained by questioning (during admission to hospital treatment, during treatment and after discharge) and detailed analysis of medical records (Form № 003/y), physical examination, general clinical study of peripheral blood.

Statistical processing of the study results was carried out by methods of variation statistics using the program «SPSS 17.0».

Results: The clinical and laboratory picture of measles in the examined patients is typical for this disease. Measles vaccinated children are significantly less likely to have fever ($p=0.001$) and Koplik's spots ($\chi^2=3.80$, $p=0.051$), the duration of fever ($p=0.001$), cough ($p=0.000$), and the length of hospital stay ($p=0.000$), as well as 3.0 times less often leukopenia is detected ($p=0.043$) and 2.0 times less often – acceleration of ESR ($p=0.023$).

Conclusions: The obtained data raise the question of expanding the explanatory work on the importance of a full course of preventive vaccination among children, as well as increasing the volume of public procurement of necessary drugs to increase public access to free vaccination.

KEY WORDS: measles, vaccination, children

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INTRODUCTION

A global strategic plan for measles and rubella control took place in the world from 2012 to 2020. Its aim was to eliminate measles by 2020 [1]. According to WHO estimated data for the period from 2000 to 2015 measles vaccination reduced the global mortality from this disease by 79 %: from 544000 cases of fatal outcomes in 2000 to 134000 – in 2015. However, the incidence of measles does not lose its relevance and, despite the possibility of effective prevention of this disease through vaccination, more than 20 million people become infected annually [2].

Outbreaks of measles periodically occur in many countries of the world, the main risk factor of which is insufficient immunological protection of the population due to factors such as increased internal and external migration, low vaccination coverage in violation of its schedules and timing, as well as unreasonable medical exemption from measles vaccination [3-7]. Mandatory vaccinal prevention, including measles, using the combined vaccine against measles, mumps and rubella, is regulated in our country by the MoH orders № 551 of 11.08.2014 «On improving the implementation of prophylactic vaccinations in Ukraine» and № 947 of 18.05.2018 «On making amendments to the calendar of prophylactic vaccinations in Ukraine». However, today Ukraine is on the list of 9 countries in the

European region endemic for measles and over the past 15 years 80 % of cases of this disease have been reported among Ukrainians [8-9]. In recent years, an increase in the incidence of measles in Ukraine was observed in 2001, 2006 and 2012. [4, 10-11]. The next epidemic outbreak of this disease was recorded in the period 2017-2019, in particular in the Poltava region, which is explained by the relatively low coverage of measles vaccination in the years preceding the rise in the incidence rate [12].

Thus, the study of the clinical and laboratory characteristics of measles, as well as the comparative characteristics of the course of this disease in vaccinated and unvaccinated children, is an urgent scientific and practical task.

THE AIM

The aim of the research is to study the clinical and laboratory characteristics of measles, as well as to compare the course of this disease in vaccinated and unvaccinated children in the Poltava region (Ukraine).

MATERIALS AND METHODS

To achieve this goal 104 patients with measles were examined: girls – 54 (51.9 %), boys – 50 (48.1 %) aged 6 months to 16

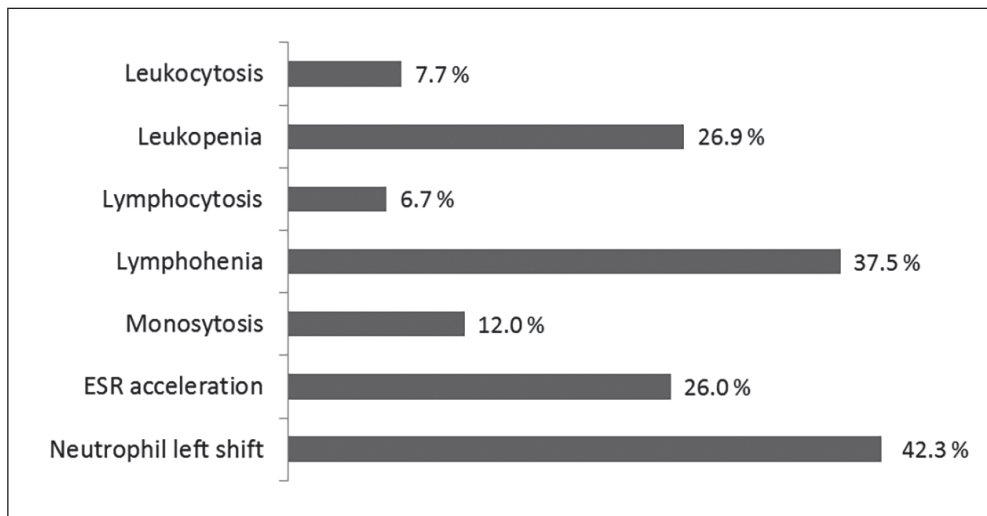


Fig. 1. An individual analysis of hemogram indicators of children with measles

Table I. Comparative characteristics of the clinical picture of measles in vaccinated and unvaccinated children

Symptoms	Groups of patients with measles		p
	I, n=27, abs. (%)	II, n=77, abs. (%)	
Maculopapular stage rash	24 (88.9)	73 (94.8)	0.372
Conjunctivitis	19 (70.4)	56 (72.7)	0.814
Koplik's spots	11 (40.7)	48 (62.3)	0.051
Dry cough	23 (85.2)	73 (94.8)	0.200
Fever	22 (81.5)	77 (100.0)	0.001

Note. The significance level was obtained using Fisher's exact test and χ^2 criterion, depending on the assumptions of the analysis.

years, average – 5.7 ± 0.4 . All patients were hospitalized in the children's infectious ward of the municipal enterprise «1st city clinical hospital of Poltava city Council» in 2017-2019. Patients' parents gave written consent to the processing of personal data.

The patient examination program included: assessment of complaints and anamnestic data obtained by questioning (during admission to hospital treatment, during treatment and after discharge) and detailed analysis of medical records (Form № 003/y), physical examination, general clinical study of peripheral blood.

Statistical processing of the findings was carried out using the program «SPSS 17.0».

To determine the central trend, the mean value and standard error of the mean value were used – in the sample $n > 100$ or the median (*Me*) with the upper and lower quartiles (*Q1-Q3*) – in the sample $n < 100$. The significance of differences in quantitative results was determined using the Mann-Whitney U-test, qualitative – by analyzing contingency tables using the exact Fisher test and χ^2 criterion depending on the assumptions of the analysis.

For all types of analysis, the differences were considered significant at $p < 0.05$, at p in the range from 0.05 to ≤ 0.1 a tendency towards significance was marked.

RESULTS

The study found that the overall clinical picture of measles in the examined children was typical for this disease. Thus, a maculopapular stage rash occurred in 97 patients (97.3 %), conjunctivitis in 75 (72.1 %), Koplik's spots in 59 (57.6 %), dry cough in 96 (92.6 %), the average duration of which was 5.6 ± 0.3 days and fever in 99 (95.2 %), the average duration is – 3.1 ± 0.2 days.

General blood counts were as follows:

- erythrocytes from 3.0 to $5.0 \cdot 10^{12}/l$, on average – 3.9 ± 0.3 ;
- hemoglobin from 80.0 to 160.0 g/l, on average – 120.8 ± 1.2 ;
- leukocytes from 1.9 to $12.6 \cdot 10^9/l$, on average 5.5 ± 0.2 ;
- ESR from 3.0 to 34.0 mm/h, on average 14.3 ± 0.8 ;
- band neutrophils from 1.0 to 35.0 %, on average 6.9 ± 0.5 ;
- segmented neutrophils from 15.0 to 78.0 %, on average 51.6 ± 1.3 ;
- monocytes from 0.0 to 14.0 %, on average 5.5 ± 0.3 ;
- lymphocytes from 6.0 to 71.0 %, on average 34.1 ± 1.4 .

The data of an individual analysis of hemogram indicators of the examined patients are presented in Fig. 1.

As shown in fig. 1, leukocytosis occurred only in 7.7 % of patients, while leukopenia was 3.5 times more likely (26.9 %), lymphocytosis was 6.7 %, lymphopenia was 5.6 times more likely (37.5 %), monosytosis was 12.0%, and ESR acceleration was 26.0 %, leukocyte shift to the left – 42.3 %. Thus, the most characteristic changes in the hemogram are leukocyte shift to the left, lymphopenia and an acceleration of ESR.

The examined patients were divided into groups in order to compare the clinical and laboratory characteristics of measles in vaccinated and unvaccinated patients:

- I – vaccinated patients, $n=27$, girls – 10 (27.0 %), boys – 17 (63.0 %) aged 6 months to 16 years, $Me=5.0$ (2.0-11.0);
- II – unvaccinated patients, $n=77$, girls – 44 (57.1 %), boys – 33 (42.9 %) aged 6 months to 16 years, $Me=4.0$ (2.0-8.0).

Taking into account the low number of patients who received the full course of measles immunization (at 12

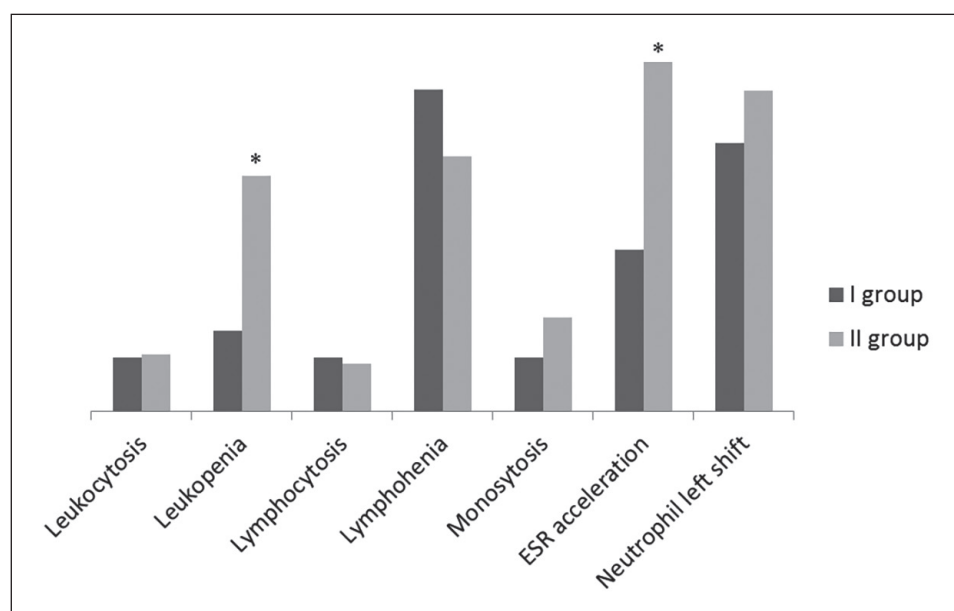


Fig. 2. Individual analysis of hemogram values for measles in vaccinated and unvaccinated children.

Note. – $p < 0,05$ (significance level obtained using Fisher's exact test and χ^2 criterion depending on the assumptions of the analysis).

Table II. Comparative characteristics of hemogram indicators for measles in vaccinated and unvaccinated children

Indicators	Groups of patients with measles		P
	I, n=27, Me (Q1-Q3)	II, n=77, Me (Q1-Q3)	
Red blood cells	4.0 (3.9-4.1)	3.8 (3.6-4.2)	0.199
Hemoglobin	124.0 (118.0-130.0)	120.0 (110.0-128.0)	0.790
Leukocytes	5.2 (4.4-7.0)	5.0 (3.7-7.0)	0.272
ESR	8.0 (5.0-15.0)	16.0 (8.0-22.0)	0.005
Band neutrophils	5.0 (3.0-8.0)	6.0 (3.0-10.0)	0.769
Segmented neutrophils	53.0 (42.0-62.0)	51.0 (45.0-61.0)	0.967
Monocytes	2.0 (2.0-3.0)	6.0 (4.0-8.0)	0.000
Lymphocytes	34.0 (24.0-45.0)	35.0 (24.0-43.0)	0.798

Note. The significance level was obtained using the Mann-Whitney test.

months and 6 years) – $n=3$, that made statistical generalization impossible, these patients were assigned to group I.

Comparative characteristics of the clinical picture of measles in patients of groups I and II are presented in table I.

As can be seen in the table I, fever was significantly less frequently observed in patients of group I – in 22 (81.5 %) versus 77 (100.0 %) in group II ($p=0.001$), and also, with a tendency towards significance, Koplik's spots – 11 (40.7 %) and 48 (62.3 %) respectively ($\chi^2=3.80$, $p=0.051$). By the frequency of other symptoms, there was no difference between the compared groups.

It should be noted that the fever in patients of group I lasted less than in II – from 0 to 6 days, $Me=1.0$ (1.0-4.0), while in II – from 1 to 7, $Me=3.0$ (1.0-5.0), $p=0.001$, the duration of cough in groups I and II was from 0 to 7 days, $Me=3.0$ (3.0-5.0) and from 0 to 14 days $Me=6.0$ (5.0-8.0) respectively, $p=0.000$.

Comparative characteristics of patients hemogram parameters of groups I and II are presented in table II.

As can be seen from the data in table II, in the hemogram of the examined patients, a significant difference between the I and II comparison groups was observed exclusively by the indicator of monocytes: 2.0 (2.0-3.0) % versus 6.0 (4.0-8.0) %, $p=0.000$, which, however, did not exceed the upper limit of normal. Other indicators were not statistically different and did not go beyond the reference values.

Then an individual comparative analysis of the hemogram data of the examined patients was carried out. The analysis revealed that significant differences in the comparison groups were observed in the frequency of registration of leukopenia and acceleration of ESR. So, in group I leukopenia was detected in 3 (11.1 %) patients, which was almost 3.0 less than in group II – in 25 (32.5 %), $p=0.043$, and acceleration of ESR – 2.0 times less often, 6 (22.2 %) and 37 (48.1 %) respectively, $p=0.023$. There was no difference in the frequency of registration of other indicators: leukocytosis – 2 (7.4 %) and 6 (7.8 %), $p=1.0$, left shift of the leukocyte formula – 10 (37.0 %) and 34 (44.2 %), $p=0.652$, monocytosis – 2 (7.4 %) and 10 (13.0 %), $p=0.727$, lymphocytosis – 2 (7.4 %) and 5

(6.5 %), $p=1.0$, lymphopenia – 12 (44.4 %) and 27 (35.1 %), $p=0.386$, respectively (Fig. 2).

When comparing the duration of inpatient treatment, it was also found that in patients of group I it was from 3 to 7 days, $Me=5.0$ (3.0-5.0), while patients of group II needed longer treatment and observation in a hospital – from 3 to 10 days, $Me=7.0$ (5.0-7.0), $p=0.000$.

DISCUSSION

As a result of our study we indicate a more severe clinical course of measles in unvaccinated children and more serious changes in laboratory parameters, which consistent with data from the scientific literature [13-15]. An increase in the length of stay in hospital was also established, which creates a significant burden on the health care system of Ukraine.

It is well known that to prevent measles outbreaks, the number of immunized people should be at least 95% [1-2]. According to official data, that the increase in the incidence of measles in the Poltava region and in Ukraine as a whole was preceded by years with a rather low coverage of the population with vaccination against this disease [4,8,12]. So, a relatively high percentage of vaccinated children in the Poltava region was observed only in 2012 and had a constant downward trend in subsequent years. During the period from 2013 to 2018, the absolute number of cases of measles registered in this region among children under 17 years old increased 13.6 times, and among adults – 24 times [12]. This disappointing situation is a consequence of the insufficient level of immunization among children, and also indicates the need for additional vaccination among adults.

However, with the joint actions of all healthcare professionals and the public, as well as appropriate financial support, in Ukraine and, in particular, in the Poltava region, there are conditions for high-quality preventive and anti-epidemic measures against measles, which meet an important strategic task related to the global elimination of this disease.

The obtained data raise the question of expanding the explanatory work on the importance of a full course of preventive vaccination among children, as well as increasing the volume of public procurement of necessary drugs to increase public access to free vaccination.

CONCLUSIONS

1. The clinical picture of measles and laboratory data in the examined patients are typical for this disease (maculopapular staged rash – 97.3 %, conjunctivitis – 72.1 %, Koplik's spots – 57.6%, dry cough – 92.6 %, fever – 95.2 %, leukocytosis – 7.7 %, leukopenia – 26.9 %, lymphocytosis – 6.7 %, lymphopenia – 37.5 %, monocytosis – 12.0 %, acceleration of ESR – 26.0%, leukocyte shift to the left – 42.3 %).
2. Measles vaccinated children are significantly less likely to have fever ($p=0.001$) and Koplik's spots ($\chi^2=3.80$, $p=0.051$), the duration of fever ($p=0.001$), cough ($p=0.000$), and the length of hospital stay ($p=0.000$),

as well as 3.0 times less often leukopenia is detected ($p=0.043$) and 2.0 times less often – acceleration of ESR ($p=0.023$).

3. The obtained data raise the question of expanding the explanatory work on the importance of a full course of preventive vaccination among children, as well as increasing the volume of public procurement of necessary drugs to increase public access to free vaccination.

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ORCID and contributionship:

Valentyna I. Ilchenko: 0000-0002-1451-442X ^{A-F}

Liudmyla M. Syzova: 0000-0002-8335-3295^{A-F}

Kateryna V. Pikul: 0000-0002-5724-4343 ^{D-F}

Iryna L. Dvornyk: 0000-0002-3660-3239 ^{D-F}

Oksana V. Muravlova: 0000-0002-1202-7206 ^{D-F}

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CORRESPONDING AUTHOR

Lyudmyla M. Syzova

Poltava State Medical University

23 Shevchenko st., 36024 Poltava, Ukraine

tel: +380662128133

e-mail: isizof@gmail.com

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ORIGINAL ARTICLE

EFFICACY OF RECREATIONAL PHYSICAL ACTIVITY FOR PERIMENOPAUSAL WOMEN WITH HYPERTENSION ONSET

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Larysa A. Ruban, Halyna M. Putiatina, Olena Ye. Hant, Pavlo B. Yefimenko, Oksana P. Kanishcheva,
Oleksii H. Honcharov, Inna A. Sasko

KHARKIV STATE ACADEMY OF PHYSICAL CULTURE, KHARKIV, UKRAINE

ABSTRACT

The aim: To examine the effect of the developed wellness program on hemodynamic indices and psychological state of perimenopausal women.

Materials and methods: The study involved 27 women aged 39 to 47 years. Inclusion criteria: recorded perimenopause, climacteric syndrome, history of episodic increase in blood pressure up to 140/85 or 150/90 mmHg. Exclusion criteria: primary hypertension, diabetes mellitus, chronic contagious, systemic, oncology diseases, and mental illnesses. Research procedure comprised tracing of the heart rate, blood pressure registration, and pulse pressure calculation. Robinson index, the coefficient of endurance according to A. Kvas formula were calculated. The assessment of psychological state was made using the Spielberger-Khanin Inventory.

Results: Multidisciplinary professionals gave classes upon wellness program at "Women's Health" school. The program included educational conversations, healthy walking, massage, psycho-corrective exercises. The dynamics of the studied parameters was analyzed after 12 weeks. Based on the anthropometric measures findings the tested women showed improvement of 6,76% weight loss; and their body mass index decreased by 6,77%. Initial numbers of Robinson index and the coefficient of endurance were above normal values. After working under the program statistically significant dynamics was observed ($p < 0,05$), although there were still signs of cardiovascular disorders. There were statistically significant positive changes Spielberger-Khanin Inventory on State Anxiety (-9,09%) and Personal Anxiety Scales (-6,96%) ($p < 0,05$).

Conclusions: Early detection of risk factors and physical activity will help to bring a vast improvement to prognosis and quality of life of perimenopausal women with hypertension onset.

KEY WORDS: recreational physical activity, psychological state, Robinson index, coefficient of endurance

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INTRODUCTION

The first signs of perimenopause in women, that is irregular period, develop after 40, however the latest scientific data show earlier onset of this period. Perimenopause in each woman involves specific physiological changes of different organs and systems associated with ovarian failure and loss of function [1-3]. Failure to produce estrogen provokes endothelial dysfunction and increased body mass index which accounts for development of hypertension. Arterial hypertension (AHT) is rated as one of the determinants of cardiovascular death. As reported by WHO cardiovascular diseases (CVD) are the leading cause of mortality in European and American countries. In the previous decades more serious epidemiological situation was more prominent among men resulting in underestimation of the significance of precursory symptoms of AHT in women. Over the last years there has been heightened interest in women's health during menopause when persistent elevation in blood pressure starts [4-7].

Prominent autonomic balance, sympathetic nervous system activation, clinical manifestations of autonomic dysfunction progress during perimenopause [8,9]. The effect of risk factors on nosogenesis and prognosis of CVD takes attention of many scientists. Psycho-emotional

stress, environmental pollutants, bad health habits, physical inactivity are associated with development of various vasculomotor, neuropsychic, endocrinous and metabolic as well as cardiovascular disorders significantly worsening not only the quality of life but also prognosis for the future [7,10,11].

From the psychosocial aspect women often associate this transition period in their life with senility. Many women may experience heavy mental workload, lack of control over their life, anxiety, confusion and emotional void. Some women seek to confront these age-related difficulties by using alcohol or drugs which aggravates the problem even more [4,10,12].

More pronounced meteorosensitivity is present in women during menopause. Typically causes of such state resulting in failure of immune system, are as follows: ill-balanced and unhealthy diet; lack of fresh air; chronic fatigue and frequent stress situations; bad health habits; unfavorable ecological setting; lack of physical activity [8,9].

The development of wellness programs comprising kinesitherapy, mindset training, massage, and partnership expansion programs play the essential role in improving the quality of life of such patients. Thus, North American Menopause Society (NAMS) experts suggest that women

having mild presentation of vasculomotor symptoms initially change their lifestyle and use drug-free aids as treatment [9]. The study by Kronenberg F., Fugh-Berman A. proved the effect of breathing techniques on sympathetic nervous system and hot flash frequency reduction by 35% and more compared to muscular relaxation [cit. 13]. Nelson H.D., Haney E., Humphrey L. et al. (2005) in their study used methods of traditional Chinese medicine such as yoga, acupuncture, herbal therapy to treat hot flash, however, the efficacy of this program was not proved [11,12].

Despite the proven effects of kinesitherapy and psychocorrection methods on the state of the body system of perimenopausal women, the studies of the efficacy of the comprehensive approach to preventing AHT by drug-free modalities were not conducted. In scientific literature there are works related to the development of wellness programs for the age category of population, aimed at improving quality of life [14-17]. Considering that worldwide working age population die from CVD, and now it is not only a healthcare problem but also an economic one, AHT prevention in perimenopausal women is subject to importance of wellness programs development [18,19].

THE AIM

To examine the effect of the developed wellness program on hemodynamic indices and psychological state of perimenopausal women.

MATERIALS AND METHODS

“Women’s Health” school was created at the premises of Kharkiv State Academy of Physical Culture. The study involved 27 women aged 39 to 47 years. Inclusion criteria: recorded perimenopause, climacteric syndrome, history of episodic increase in blood pressure up to 140/85 or 150/90 mmHg. Exclusion criteria: primary hypertension, diabetes mellitus, chronic contagious, systemic, oncology diseases, and mental illnesses. The research related to human use has been complied with all the relevant national regulations and institutional policies, principles of the Helsinki Declaration, adopted by the General Assembly of the World Medical Association (1964-2000), the Council of Europe Convention on Human Rights and Biomedicine (1997).

Detailed analysis of life record data showed that body weight gain, decrease in physical activity, eating disorders, lability of nervous system, response to weather condition changes in the form of headaches and elevation in blood pressure were present in all women over the last 3 to 5 years. Research procedure comprised tracing of the heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) registration, and pulse pressure (PP) calculation. Robinson index and the coefficient of endurance were calculated. The assessment of psychological state was made using the Spielberger-Khanin Inventory.

Robinson index (RI) is used to assess the level of metabolic and energy processes in the body. It is representative of the level of hemodynamic load on the cardiovascular system

(CVS). RI shall be calculated using the following formula: $RI = HR_r \times SBP / 100$, where HR_r – is resting heart rate, bpm; SBP – systolic blood pressure, mmHg. Average value of the RI is 81 to 90 c.u., more than 111 c.u. – is impaired regulation of CVS.

Coefficient of endurance (CoE) is a descriptor of CVS condition level while performing physical activity. It is determined from A. Kvas formula: $CoE = (HR_r \times SBP \times 10) / PP$, where HR_r – is resting heart rate, bpm; SBP – systolic blood pressure, mmHg; PP – pulse pressure, mmHg. Normative value is equal to 16 c.u., an increase in CoE indicates CVS deconditioning.

Spielberger-Khanin inventory enables differentially assessing anxiety according to two subscales both as trait and state associated with the current situation. State anxiety scale (S-anxiety) is a descriptor of individually experienced emotions at that moment. Trait anxiety scale (T-anxiety) is a constitutional trait accounting for proneness to perceive threat over a wide range of situations. The results of survey were recorded in points, and their interpretation were as follows: less than 30 points – mild anxiety, 31-45 point – moderate anxiety, 46 and more points – severe anxiety.

Statistical processing was carried out using STATISTICA 13.0 (StatSoft) statistics package.

RESULTS

The operation of “Women’s Health” school was aimed at teaching recreational physical activity to women for the purpose of improving their quality of life [20,21]. A multidisciplinary team was created to work upon the program. The team included a physical therapist, teacher, rehabilitation coach, psychologist, massage therapist.

While making the program by physical therapist, the principles of physical therapy and pedagogical cooperation, specifically affordability, integrity and multidisciplinary, were followed [22]. The program provided conversations on health promotion, healthy walking, peer massage of neck and collar area, psychocorrective exercises.

Educational part of the program included conversations with women to form their strong beliefs about the need for lifestyle changes, eating behavior correction and fulfilling recreational physical activity requirements; information on menopause, structure and functions of body systems, pathogenesis and medical and social consequences of CVD, meteosensitivity prevention.

At the beginning of the program 3 to 5 women wearing light clothing supervised by the rehabilitation coach walked every other day in the park area during 45 minutes, first at a slow pace 60 to 70 steps per minute at a distance of 500 to 700 m. Starting from day 10, the route of 800 to 1500 m was set, walking pace was gradually increased up to 80 - 90 steps per minute and lasted for 45 to 60 minutes. It was recommended to control breathing. Inhale when making the 2 steps, exhale during the next 3-4 steps. Sessions should be regular, since cumulative positive effect subsides after their cancellation [7,10].

The massage therapist ran classes with women on practical mastery of peer massage session [23]. Peer massage technique includes the following maneuvers:

Table I. Dynamics of anthropometric measures of perimenopausal women

parameter	women (n=27)		p
	initial data	data after the program	
	$\bar{X} \pm m$	$\bar{X} \pm m$	
height, cm	165,00±2,37	165,00±2,37	>0,05
weight, kg	74,00±2,18	69,00±1,43	>0,05
BMI, kg/m ²	27,18±0,94	25,34±0,67	>0,05

Table II. Positive changes of hemodynamic indices of perimenopausal women with AHT onset

parameter	women (n=27)		p
	initial data	data after the program	
HR, bpm	87,08±1,31	78,55±1,56	<0,05*
SBP, mm Hg	138,13±1,59	127,78±1,39	<0,05*
DBP, mm Hg	87,22±0,74	74,73±0,63	<0,05*
PP, mm Hg	50,91±0,85	53,05±0,76	<0,05*

Note: * statistically significant differences in the dynamics of values

Table III. Dynamics of systolic heart function values of the perimenopausal women with AHT onset

parameter	women (n=27)		p
	initial data	data after the program	
Robinson index, c.u.	120,28	100,37	<0,05*
Coefficient of endurance, c.u.	17,1	14,8	<0,05*

Note: * statistically significant differences in the dynamics of values

1. Palmar surface of both hands laid on the neck and shoulder girdle; and a light spiral rubbing of the neck and shoulder girdles was performed with light force. After that palms moved to scapular and interscapular region. This rubbing was performed for 30 to 40 sec.

2. Two palms laid on shoulder girdle muscles, then one of them smoothly stretch while the other goes opposite way.

The distance between palms shall be 5 to 6 cm. Quantity – 2 to 3 times each side.

3. Shoulder girdles were kneaded starting from neck and to the shoulder joints using heel of the hand; 2 to 3 passed each side; interscapular region from inferior angle of scapula up to neck-shoulder point, 2 to 3 passes; from medial border of scapula towards shoulder joints, 2 to 3 passes each side.

4. At the end 4 to 5 active moves on maximum retraction and protraction of scapula were performed. The whole session took 4 to 5 minutes [23].

The psychocorrective unit involved running 9 sessions lasting 90 minutes and doing exercises without assistance. Common activity; work in subgroups (3 to 4 women); and individual work were used. Psychocorrective unit sessions started with motivational fun games aimed at forming the atmosphere of trust within the group [24,25]. Art therapy and body-oriented therapy methods were used during psychocorrective sessions. Art therapy and body-oriented therapy methods incited women to speak through their unconscious emotions.

Respiration management was used to decrease anxiety level of the women. All respiration exercises were divided into sets: 8 repetitions per one set. Stimulating respiration exercises were performed seated or in the upright position; relaxation ones were done in a supine position. The number of repetitions increased gradually, and the quality of respiratory load also changed. To promote their mental state, the women were offered to do respiration exercises stimulating sympathetic division of nervous system: focus on inhalation (loudly, deeply), exhalation (quietly). To relax their current state women were taught diaphragmic respiration. Also, hypnosis and neurolinguistic techniques were used during psychocorrective sessions to form attitudes to preserve and strengthen mental health.

After 12 weeks the physical therapist analyzed the dynamics of the studied parameters. The following changes took places within the group of persons at “Women’s Health” school. Thus, according to the results of anthropo-

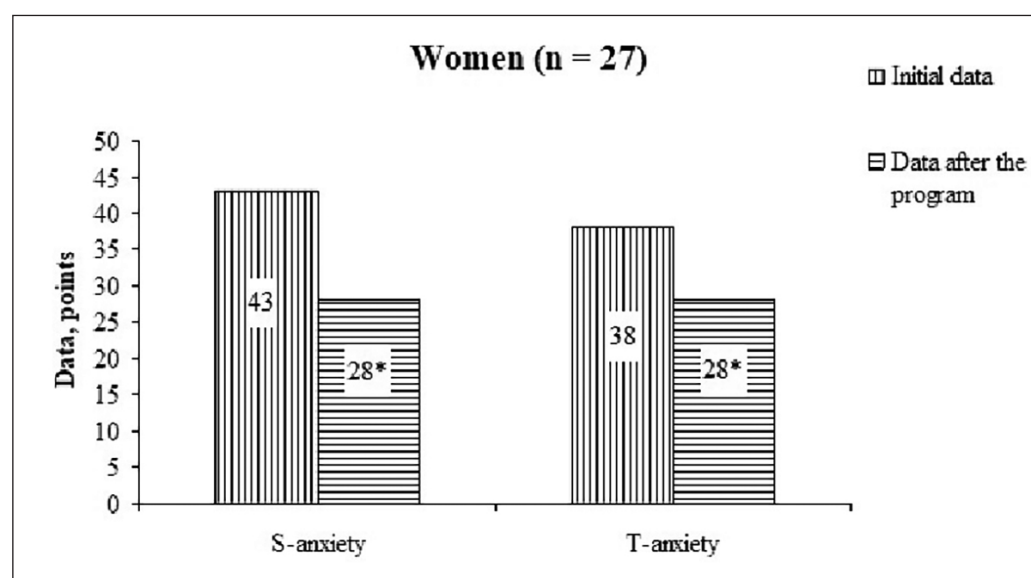


Fig. 1. Dynamics of medians of indicators of the psychological state of perimenopausal women
Note: * statistically significant differences in the dynamics of values

metric measures the tested women showed improvement of 6,76% weight loss; and their body mass index decreased by 6,77% (table I).

Although no statistically significant dynamics was observed ($p > 0,05$), after wellness program perimenopausal women had a pronounced tendency to decrease body weight and BMI.

After working upon the program an improvement in all hemodynamic indices was observed. Thus, HR values significantly decreased by 9,80%, SBP by 7,49%, DBP by 14,32% ($< 0,05$). PP value statistically significantly increased by 4,20% ($< 0,05$) (table II).

Based on the findings of hemodynamic indices for quantitative assessment of systolic heart function, the Robinson index was calculated and coefficient of endurance (CoE) according to Kvas formula was estimated. At initial examination mean value of the Robinson index in the group of women was 120 c.u., which is 1,28 times higher than the normal value and is declarative of impaired regulation of cardiovascular system. CoE according to the Kvas formula during the initial calculation showed weakening of cardiovascular system: while the normal value is 16 c.u., among the tested women this parameter got the value equal to 17,1 c.u. (table III).

Analysis of the dynamics showed positive changes: Robinson index and CoE decreased by 16,55 and 13,45% respectively. After working upon the program statistically significant dynamics was observed ($p < 0,05$), although there were still signs of cardiovascular disorders.

Considering the fact that psychological disorders are among compromising factors of AHT progress and adversely affect cardiovascular performance, the Spielberger-Khanin inventory on trait (T-anxiety) and state (S-anxiety) anxiety scales was conducted among perimenopausal women. When assessing psychological state of the tested persons, statistically significant increase in state and trait anxiety level according to Spielberger-Khanin inventory was determined compared to normal values ($p < 0,05$). After completing the wellness program, there was a statistically significant dynamics of Spielberger-Khanin Inventory on State Anxiety Scale (-9,09%) and (-6,97%) on Trait Anxiety Scale ($p < 0,05$) (fig.1).

Thus, there were positive changes in hemodynamic indices and psychological state of perimenopausal women with AHT onset affected by the developed wellness program.

DISCUSSION

Perimenopausal women experience persistent increase in arterial blood pressure. The issues of the quality of life of perimenopausal women are poorly covered in the literature, inadequate attention is given to the development of wellness programs, occasionally wellness programs for the elderly can be found. Development of recreational physical activity program should be aimed at meeting the needs and components to improve the quality of life of the women during this period. Health and wellness program should include physical activity, correction of eating behavior,

psychocorrective exercises, behavioral components such as meteosensitivity conditioning which is shown in our study [6,8-10,19].

Perimenopausal women's data obtained during the study showed the disposition towards increase in weight and BMI emphasizing the importance of this risk factor for AHT progression. An increase in impaired regulation of CVS was indicated, which is subject to the predominance of increased BP values. An increase in anxiety level according to Spielberger-Khanin Inventory was indicated. All of the above data point to a decrease in the quality of life of perimenopausal women.

To prevent AHT it is necessary to do moderate aerobic physical activity. Prospective study shows that CVD risk is 30% higher for physically non-active middle-aged people. The study confirmed that regular moderate physical activity promotes the development of adjustment reactions, immunity to environmental exposure, increased metabolism in tissues, coping with hypoxia, heart function economy [13-17].

Nelson H.D. et al., Agmon, M. et al. [11], studied the effect of healthy walking and fitness on clinical condition of the women having symptoms associated with menopause. The analysis of the positive changes in hemodynamic indices and parameters of psychological state of the women from "Health school" confirmed the results of the similar study.

Thus, currently there is no framework defining the development of wellness programs which could detect preterm hemodynamic and psychological changes in this category of women. Early detection of risk factors and targeted influence on physical activity and psychological state will help to significantly improve the prognosis and quality of life of perimenopausal women.

CONCLUSIONS

Based on the study, the changes in hemodynamic indices and psychological state were detected in the perimenopausal women with AHT onset, which confirms the risk of CVD progression. The dynamics of the above parameters was statistically significantly positive: the general state of health improved, psychological state recovered, and the tolerance to physical and psychological stress has increased.

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ORCID and contributionship:

Larysa A. Ruban: 0000-0002-7192-0694 ^{A,D,F}
 Halyna M. Putiatina: 0000-0002-9932-8326 ^{D,F}
 Olena Ye. Hant: 0000-0001-7729-4914 ^{B,C}
 Pavlo B. Yefimenko: 0000-0003-4674-6919 ^{B,C}
 Oksana P. Kanishcheva: 0000-0002-5030-5318 ^{B,C}
 Oleksii H. Honcharov: 0000-0002-2012-6298 ^{B,C,E}
 Inna A. Sasko: 0000-0002-9817-4468 ^{B,C}

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CORRESPONDING AUTHOR

Larysa A. Ruban

Kharkiv State Academy of Physical Culture
 99 Klochkivska st., 61058 Kharkiv, Ukraine
 tel: +380502468981
 e-mail: slarisaruban@gmail.com

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ORIGINAL ARTICLE

AFFECTIVE-COGNITIVE INDICATOR OF EMOTIONAL INTELLIGENCE FORMEDNESS IN HIGH SCHOOLERS WITH INTELLECTUAL DISABILITIES

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Olha O. Babiak¹, Ivan M. Okhrimenko², Natalia A. Lyakhova³, Andrii V. Lapin¹, Anna V. Zamsha¹, Kyrilo Yu. Parkhomenko⁴¹MYKOLA YARMACHENKO INSTITUTE OF SPECIAL EDUCATION AND PSYCHOLOGY OF THE NATIONAL ACADEMY OF EDUCATIONAL SCIENCES OF UKRAINE, KYIV, UKRAINE²NATIONAL ACADEMY OF INTERNAL AFFAIRS, KYIV, UKRAINE³POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE⁴KHARKIV MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KHARKIV, UKRAINE

ABSTRACT

The aim is to determine the peculiarities of the affective-cognitive indicator of emotional intelligence in high schoolers with intellectual disabilities.

Materials and methods: The study, which was conducted during 2019-2020, involved 76 high schoolers in the age of 14-17 who studied in the 7-9th grades in Kyiv schools (Ukraine) (45 high schoolers with intellectual disabilities and 31 high schoolers with normative development). The diagnosis of the high schoolers' ability to perceive, understand and identify emotions; to assimilate emotions in thoughts, to stimulate thought processes with the help of emotions was carried out. The Mayer-Salovey-Caruzo Emotional Intelligence Test, adapted for children with intellectual disabilities, was used.

Results: Insufficient formedness of the affective-cognitive indicator of emotional intelligence in high schoolers with intellectual disabilities has been determined: a decrease in the level of perception of emotions by expression; superficial perception of expressive features, vagueness of ideas about them; insufficient differentiation of the constituent elements of the emotional model; difficulties of orientation in a set of emotional signs of different modalities; lack of understanding of social emotions and the content of a moral act.

Conclusions: It has been proven that the low state of formedness of the affective-cognitive indicator of emotional intelligence results in significant difficulties that arise during interaction with each other, and this affects primarily the further socialization and integration into society.

KEY WORDS: emotional intelligence, emotionality, affective-cognitive indicator, intellectual disabilities

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INTRODUCTION

New tasks of modern education reform in relation to the teaching and upbringing of high schoolers with special educational needs require the formation of the foundations of life competence of individuals with intellectual disabilities [1-6]. One of the key determinants of the success of social adaptation of persons in this category is the formedness level of their affective-cognitive indicator of emotional intelligence. The study of the affective-cognitive indicator is a new segment in the research of emotional intelligence of an individual with special educational needs. The importance of solving the problem of emotional development is stipulated by the growing number of children with intellectual disabilities, who are officially diagnosed with low levels of cognitive processes, behavioural disorders, maladaptation in the educational and social environment [7-14].

Determining the directions of formation of affective-cognitive indicator of emotional intelligence in high schoolers with intellectual disabilities makes it possible to outline the prerequisites for their becoming as individuals, their success-

ful inclusion in social life and ability to adapt to changing social environment.

In view of this, first of all it is necessary to characterize the concept of emotional intelligence, which is considered by scientists as ability, set of abilities, group of mental abilities, capability, regulator, indicator, factor, integral category, cognitive capability, psychological phenomenon, resource, cognitive-personal composition, etc. [15-18].

Therefore, it should be noted that the main attention in the research of emotional intelligence was paid to the study of its psychological structure and relationships with other intrapersonal manifestations. Less studied are the issues of formation of the affective-cognitive indicator of emotional intelligence, namely: perception, recognition and understanding of one's own emotions, emotions of other people at different age stages of mental development of a personality. In addition, the available research does not provide a clear understanding of psychological conditions, peculiarities, factors, mechanisms of formation of affective-cognitive indicator of emotional intelligence in high schoolers with intellectual disabilities.

THE AIM

The aim of this study is to determine the peculiarities of the affective-cognitive indicator of emotional intelligence in high schoolers with intellectual disabilities.

MATERIALS AND METHODS

The vision of emotional intelligence as a set of abilities that form an affective-cognitive construct requires a certain specificity of the organization of the ascertaining study. Thus, in order to identify the formedness of affective-cognitive indicator of emotional intelligence of high schoolers with intellectual disabilities, we conducted the experimental research, which involved 76 high schoolers in the age of 14-17 who studied in the 7-9th grades in Kyiv schools (45 children with intellectual disabilities and 31 high schoolers with normative development). The study in comparative terms makes it possible to more thoroughly identify the specific peculiarities of the affective-cognitive indicator of emotional intelligence in high schoolers with intellectual disabilities. At the same time, the performance of diagnostic tasks by children with normative development serves as an indicator of the age norm.

The study of the formedness of affective-cognitive indicator of emotional intelligence in high schoolers with intellectual disabilities was carried out in several stages according to certain criteria, which allowed determining the levels of formedness of affective-cognitive indicator of emotional intelligence of high schoolers with intellectual disabilities. The first stage involves the diagnosis of the ability to perceive, understand and identify emotions. This stage involved the usage of MSCEIT v. 2.0 (The Mayer-Salovey-Caruzo Emotional Intelligence Test), adapted for children with intellectual disabilities; the second stage includes the diagnosis of the ability to assimilate emotions in thoughts, to stimulate thought processes with the help of emotions using the method of N. Hall [19].

We used the rank correlation coefficient according to the two-sided Student's t-test (for independent, unrelated samples) to compare the results of the study. The results were considered reliable at $p < 0.05$.

The study was performed in accordance with the requirements of the Code of Ethics of Scientists of Ukraine, approved by the Resolution of the General Meeting of the National Academy of Sciences of Ukraine (Protocol No. 2 of April 15, 2009). According to its provisions, the members of the scientific community are guided by the rules of ethical conduct and professional communication; respect the principles, values, norms, rules, and conditions of academic honesty in their activities. The preliminary consent to participate in the research was obtained from all respondents.

RESULTS

The peculiarities of the affective-cognitive indicator were studied in the course of the research i. e. the perception of expressive signs of emotions, types of perception of expres-

sion. To this end, the ability to adequately recognize and verbalize the emotional state presented in the schematic image was studied.

The quantitative and qualitative analysis of the results of the study revealed that high schoolers with intellectual disabilities (ID) have mostly medium and low levels of expression perception, the number of established expressive traits is much lower (62.5%) than in high schoolers with normative development (ND) (80 %). The results of the study of the level of perception of expressive signs of emotions are presented in Table I.

The Student's t-test was used to assess the significance of differences between the levels of perception of expression found during the comparative analysis of the studied groups of high schoolers. Numerical values of the Student's t-test between the groups of children (ID and ND) are statistically significant: between the 7th grades high schoolers with ND and ID ($t = 3.63$ at $p \leq 0.05$); between the 9th grade high schoolers with ND and ID ($t = 3.23$ at $p \leq 0.05$), as well as in the groups of high schoolers: between the 7th grade high schoolers with ND and the 9th grade high schoolers with ND ($t = 3, 09$ at $p \leq 0.05$); between the 7th grade high schoolers and the 9th grade high schoolers with ID ($t = 2.05$ at $p \leq 0.05$).

Thus, the perception of expressive signs of emotions in high schoolers with intellectual disabilities is at a lower level of development, compared with the age norm. The high schoolers with ID are completely unable to perceive, recognize and understand their own emotions and the emotions of other people, they explain one emotion through another (difficulties at the stage of categorization are primarily related to the underdevelopment of mental activity, memory and imagination); lack of self-awareness and awareness of another person (indicating a low level of value of another person for adolescents with ID).

In contrast to the high schoolers with intellectual disabilities, the children with normative development correctly interpreted the meaning of the emotional pictogram focusing on their emotional experience; they could explain the presence of those signs of expression that indicated the emotion. There was a statistically significant difference in groups between the 7th grade high schoolers: between the pre-schoolers with ND and the high schoolers with ID ($t = 4.19$ at $p \leq 0.05$). The same was seen in the groups between the 9th grade high schoolers: between the children with ND and ID ($t = 2.79$ at $p \leq 0.05$); there was also a significant difference in the groups between the 7th grade high schoolers with ND and the 9th grade high schoolers with ND ($t = 1.57$ at $p \leq 0.05$).

Analysing the level of understanding of the expressive signs of high schoolers' emotions, significant differences were revealed in this indicator between the children with ND and high schoolers with ID. Three levels were identified based on the analysis of the content of understanding of emotions by high schoolers with ID: high, medium and low (Table II).

There was a statistically significant difference in the groups between the 7th grade high schoolers with ND and the 9th grade high schoolers with ND ($t = 1.32$, $p \leq 0.05$).

Table I. Comparison of the levels of expression perception in the high schoolers with ND and ID, %

Grade	The level of expressive traits perception					
	High schoolers with normative development			High schoolers with intellectual disabilities		
	High level	Medium level	Low Level	High level	Medium level	Low level
7th grade	27	51.5	21.2 %	10	46.7	43.3
9th grade	47	41.2	11.8	11.1	61.1	27.3

Table II. Comparison of the levels of understanding of expressive signs of emotions in the high schoolers with ID and ND, %

Grade	The level of understanding of expressive signs of emotions					
	High schoolers with normative development			High schoolers with intellectual disabilities		
	High level	Medium level	Low Level	High level	Medium level	Low level
7th grade	37.3	55.4	7.3	10.7	46.4	42.9
9th grade	39.5	58.8	1.8	13.6	54.6	31.8

Table III. Comparison of the levels of emotional identification in the high schoolers with ID and ND, %

Grade	The level of emotional identification					
	High schoolers with normative development			High schoolers with intellectual disabilities		
	High level	Medium level	Low Level	High level	Medium level	Low level
7th grade	37.3	61.5	1.2	17.8	39.3	42.9
9th grade	39.5	59.8	0.7	13.6	59.1	27.3

The peculiarities of emotional identification, the peculiarities of correlation of emotion expressive signs were studied in the course of performing experimental tasks. The analysis of experimental data showed that the establishment of the emotion identification was common to all categories of children. At the same time, the high schoolers with ND independently organized activities, and the high schoolers with ID did not begin to perform tasks due to the narrowing of the scope of their perception. The results of the study of the levels of identification of emotions in the high schoolers with ID and ND are presented in Table III.

In our opinion, the causes of difficulties in identifying emotions can be justified by the peculiarities of the mechanisms of identification of emotions in high schoolers with intellectual disabilities, they do not have adequate terms to describe the emotions of another person, children are able to identify an emotion based on only one source of information i. e. the situation in which the character found himself or his facial expression. On the other hand, the identification of emotions is influenced by the shortcomings of intellectual functions, as well as the conditions in which the high schoolers with ID live and are brought up.

The method of mathematical statistics revealed a significant difference in the groups between the 7th grade high schoolers: between the high schoolers with ND and ID ($t = 1.63$ at $p \leq 0.05$). A significant difference was revealed in the groups of the 9th grade high schoolers, in particular between the high schoolers with ND and ID ($t = 2.67$ at $p \leq 0.05$). There was a significant difference between the groups of the 7th grade high schoolers with ND and the 9th grade high schoolers with ND ($t = 2.44$ at $p \leq 0.05$), between the 7th grade and the 9th grade high schoolers with ID ($t = 2.44$ at $p \leq 0.05$).

DISCUSSION

The theoretical basis of our theoretical and experimental research was the position that increased emotional competence improves psychological and physiological well-being, which is a necessary condition for socialization and integration into society [6, 20-27]. In support of the position of scientists, our study showed that high schoolers with intellectual disabilities are characterised by insufficiently developed emotional competence, in contrast to high schoolers with normative development.

Our study is also a supplement to the conclusions made by Chetveryk-Burchak [28] that the mechanisms of emotional intelligence, which are based on a positive or negative attitude towards the object, the focus on the object or away from it and the modality of emotion affect the life-sustaining activities of the individual.

At the same time, our results complement the scientific research by Megías, Gutiérrez-Cobo, Fernández-Berrocal, Cabello, & Gómez-Leal [12] in terms of the fact that emotional intelligence is associated with perceptual, cognitive processes, as well as their physiological characteristics. It should be noted that the high schoolers with intellectual disabilities have mostly low and medium levels of perception, understanding of expressive signs and arbitrary expression of emotions of different modalities, which affects the formation of their personality and successful inclusion in social life and ability to adapt to changing social environment.

CONCLUSIONS

The analysis of the psychological literature showed gaps in the study of emotional intelligence in high schoolers with

intellectual disabilities. This became the basis of our experimental study. Thus, it was found that the high schoolers with intellectual disabilities are characterised by insufficiently formed affective-cognitive indicator of emotional intelligence; the low state of this indicator formedness results in significant difficulties that arise during interaction with each other.

The results of our study give the right to ascertain the existing problems in the personal development and emotional intelligence of high schoolers with intellectual disabilities. They feel unprotected, have emotional anxiety and are therefore highly dependent on their surroundings. The presence of deep originality of emotional and volitional development largely determines the low level of affective-cognitive indicator formedness, being one of the reasons for low social activity and low level of social adaptation of children in this category. This situation encourages the delineation of areas of correctional work on the formation of affective-cognitive indicator of emotional intelligence of adolescents.

We consider it promising to study the levels of emotional intelligence formedness in high schoolers with intellectual disabilities of middle and senior school age in secondary schools in comparison with their healthy peers.

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ORCID and contibutionship

Olha O. Babiak: 0000-0001-6337-1202^{A,E}

Ivan M. Okhrimenko: 0000-0002-8813-5107^{B,F}

Natalia A. Lyakhova: 0000-0003-0503-9935^{A,C}

Andrii V. Lapin: 0000-0003-1717-7892^{B,D}

Anna V. Zamsha: 0000-0002-8843-035X^{E,F}

Kyrylo Yu. Parkhomenko: 0000-0002-0004-2417^{B,F}

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Ivan M. Okhrimenko

National Academy of Internal Affairs, Kyiv, Ukraine

tel: +380679903905

e-mail: ivango-07@ukr.net

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ORIGINAL ARTICLE

SURGICAL TREATMENT IMMEDIATE RESULTS OF LOWER LIMBS CHRONIC CRITICAL ISCHEMIA IN SIMULTANEOUS DIRECT AND INDIRECT REVASCULARIZATION

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Mykhailo M. Lopit, Vasily I. Rusin, Patricia O. Boldizhar, Fedir V. Gorlenko, Olexander M. Kochmar

HIGHER STATE EDUCATIONAL ESTABLISHMENT OF UKRAINE "UZHGOROD NATIONAL UNIVERSITY", UZHGOROD, UKRAINE

ABSTRACT

The aim: To assess the immediate results of autovenous femoral-tibial shunting in combination with rotary osteotripsy of the tibia by studying changes in the transcutaneous pO_2 tension in the tibia and foot tissues depending on the revascularization of the tibial arteries.

Materials and methods: We analyzed the treatment of 69 patients with obliterating atherosclerosis of the vessels of the lower extremities. According to the degree of ischemia of the lower extremities, the patients were distributed as follows: III A degree of ischemia - 20 (29%), III B - 28 (40.6%), IV - 21 (30.4%) patients.

All patients had atherosclerotic lesions of the popliteal-tibial segment of the great arteries with preserved central blood flow in the aorto-iliac segment.

Results: Occlusion of the anterior tibial artery was recorded significantly more often than the peroneal artery (72% and 42%, respectively, $p = 0.05$), but with the same frequency compared to the posterior tibial artery (68%, $p = 0.61$).

The lesion of two or three arteries of the lower leg was recorded more often than occlusion of one ($n = 51$; 73.9% and $n = 18$; 26.1%). Occlusive-stenotic lesion of the popliteal artery was observed in 54 (78.2%) patients.

The highest incidence of lesions of the tibial arteries was observed in the basin of the anterior tibial artery in 28 (40.6%) patients. Combined lesions of the anterior tibial artery and posterior tibial artery were diagnosed in 19 (27.5%) patients. Limited lesions of the posterior tibial artery were found in 15 (21.7%) patients. The combination of lesions of the posterior tibial artery and peroneal artery was diagnosed in 7 (10.2%) patients.

Conclusions: 1. The patency of the femoral-tibial autovenous shunt during the year was 71%. High limb amputation was performed in 29% of patients.

2. According to CT data, the localization of trophic changes on the foot during critical ischemia of the lower extremities corresponds to the affected segment of the arterial angiosome, which supplies the corresponding area with blood.

3. After femoral-tibial autovenous bypass grafting, the highest levels of transcutaneous oxygen tension were observed in the basin of the posterior tibial artery and peroneal artery, and the lowest indicators of transcutaneous oxygen tension were observed in the basin of the anterior tibial artery.

4. During femoral-tibial autovenous shunting operations in combination with rotary osteotripsy, the transcutaneous oxygen tension indices increased threefold in the angiosomal basin of the posterior tibial artery and peroneal artery, and twofold in the angiosomes of the dorsum of the foot and sole.

KEY WORDS: chronic ischemia of the lower extremities, distal autovenous femoral-tibial shunting, transcutaneous oxygen tension, angiosomal theory

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INTRODUCTION

Treatment of occlusive diseases of the arteries of the lower extremities is one of the urgent and difficult tasks of surgery. Reconstructive surgery is associated with a high risk, and in 25-40% of patients it is impossible to perform the intervention due to the prevalence of the occlusive process [1-4]. In the absence of an adequate distal vascular supply, the development of purulent-necrotic processes in the affected limb and the presence of contraindications to reconstructive surgery, conservative therapy or amputation remain the only treatment techniques [1-4]. For these patients in complex treatment, it is possible to perform indirect methods of revascularization - rotary osteotripsy of the tibia (ROT) - as an alternative to the amputation of the lower limb [2-5].

Theoretically, for the onset of a full-fledged effect after ROT surgery, months are required, during which collateral circulation will gradually develop. Rotary osteotripsy of the tibia increases possibilities of limb salvage or performance of more favorable resection operations on the foot in patients with IV grade limb ischemia [2,3,6].

However, some authors note the effect of revascularization osteotripsy almost immediately after surgery, which is manifest both clinically and by an increase of the transdermal pO_2 [2-5]. Rotary osteotripsy of the tibia can be performed alone or in combination with reconstructive interventions or sympathectomy. The positive effect after combined treatment is more significant and longterm in patients with distal forms of obliterating atherosclerosis [4, 5, 7].

Considering the above, we used a combination of direct and indirect techniques of treatment in patients with distal forms of atherosclerosis to improve the state of the limb microvasculature in order to decrease the number of post-operative thrombosis and amputations. The combination of direct and indirect revascularization technics provides an improvement in the state of the inflow and outflow pathways, a decrease in the thrombosis incidence in the postoperative period and an increase of the limb salvage rate [7,8,9].

THE AIM

To assess the immediate results of autovenous femoral-tibial shunting in combination with rotary osteotrepation of the tibia by studying changes in the transcutaneous pO₂ tension in the tibia and foot tissues depending on the revascularization of the tibial arteries.

MATERIALS AND METHODS

We analyzed the treatment of 69 patients with obliterating atherosclerosis of the lower extremities vessels. The patients were hospitalized at the Department of Vascular Surgery of the Transcarpathian Regional Clinical Hospital n.a. Andrey Novak from 2015 to 2020yy. According to the degree of ischemia of the lower extremities, the patients were distributed follows groups: III A degree - 20 (29%), III B - 28 (40.6%), IV degree - 21 (30.4%) patients.

In the demographic structure men dominated - 64 (92.7%) patients. The average age of women (66.1 ± 5.4) was almost five years higher than the average age of men (60.9 ± 8.6).

All patients had atherosclerotic lesions of the popliteal-tibial segment of the magistral arteries with preserved central blood flow in the aorto-iliac segment.

Trophic changes in the skin of the foot were observed in 30.4% of patients. Limited by a few toes necrotic changes prevailed - 42.9%, most often I and IV toes were affected. Concerning concomitant diseases, the following pathologies were diagnosed: ischemic heart disease - 45 (65.2%) cases, arterial hypertension - 51 (74%), chronic cerebrovascular insufficiencies II - III degree - 23 (33.3%) cases, diabetes mellitus 25 (36.2%), erosive and ulcerative-erosive lesions of the gastrointestinal tract - 37 (53.6%), chronic obstructive pulmonary diseases - 19 (27.5%).

The diagnostic program included ultrasound examination (US) - Doppler sonography, multispiral computed tomography with contrast enhancement, determination of the regional perfusion index based on the measurement of transcutaneous oxygen tension (TcPO₂) in the basin of the anterior tibial artery, posterior tibial artery, peroneal artery and arteries of the foot in the postoperative period after three months.

For the statistical analysis of the mean values of the TcPO₂ index in patients before and after operative interventions, a t-test paired two-sample for means was used. For the TcPO₂ index changes we used the percentage

ratio of the regional tissue perfusion index before and after surgery in relation to the norm. Microsoft Excel 2019 was used to analyze the data.

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

I group - 34 patients who underwent distal autovenous femoral-tibial bypass grafting in combination with rotary osteotrapation of the tibia.

II group - 35 patients who underwent distal autovenous femoral-tibial bypass grafting.

RESULTS

Contrast-enhanced multispiral computed tomography and ultrasound examination analysis allowed us to identify the following types of distal arterial lesions:

- occlusive-stenotic lesions of the popliteal artery, patent arteries of the lower extremity;
- occlusive stenotic lesion of the popliteal artery, occlusion of 1-2 arteries of the lower extremity;
- diffuse lesion of the popliteal-tibial segment with patency and / or with the absence of patency of the one tibial artery;

Occlusion of anterior tibial artery was recorded significantly more often than peroneal artery (72% and 42%, respectively, $p = 0.05$), but with the same frequency as compared with posterior tibial artery (68%, $p = 0.61$). The involvement of the foot arteries in the process was found in 21 (30.4%) patients.

The occlusion of two or three arteries of the lower leg was recorded more often than occlusion of one ($n = 51$; 73.9% and $n = 18$; 26.1%). Occlusive-stenotic lesion of the popliteal artery was observed in 54 (78.2%) patients.

The highest incidence of lower leg artery lesions was observed in the anterior tibial artery basin in 28 (40.6%) patients. Combined lesions of anterior tibial artery and posterior tibial artery were diagnosed in 19 (27.5%) patients. Limited posterior tibial artery lesions were found in 15 (21.7%) patients. The combination of posterior tibial artery and peroneal artery lesions was diagnosed in 7 (10.2%) patients.

Necrobiotic changes in the foot were most often observed on the dorsum of the foot distal parts and toes - 11 (52.4%) patients. Nine (42.9%) patients had necrosis on the plantar surface of the foot in the projection of the 2-5 metatarsal bones. In one patient (4.8%) trophic changes took place in the heel area.

The indices of the ankle pressure index ranged from 0.24 to 0.4 and directly proportionally depended on the level and extent of atherosclerotic alteration, ranged from 0.42 ± 0.15 , in III B degree - 0.38 ± 0.12 and in IV degree - 0.24 ± 0.12 .

A total of 69 operations were performed using direct revascularization (table I).

As can be seen from the table, posterior tibial artery (34.8%), anterior tibial artery (26.1%), peroneal artery (21.7%) and tibioperoneal trunk (17.4%) were used most often for tibial bypass grafting.

Table I. Distribution of patients according to the localization and technique of the distal anastomosis

Artery	Distal anastomosis	
	«end to end»	« end to side »
Anterior tibial artery	17	1
Posterior tibial artery	20	4
Peroneal artery	15	-
Tibioperoneal trunk	8	4
Total	60	9
	69	

In this case, the advantage in the formation of the distal anastomosis was given to the “end-to-side” technique (87%).

The immediate results of direct revascularization and ROT were assessed within three months on the basis of changes in clinical symptoms, which were identified during examination before surgery, as well as the presence or absence of postoperative complications. The permeability of the reconstruction zone with direct methods of revascular-

ization in the first three months was 69.6%. Reoperations were performed in 21 (30.4%) patients due to shunt thrombosis. In 20 patients from this group amputations were at the lower third level of the thigh, and only in one cause there was technical possibility to restore the blood flow.

Thrombosis after anterior tibial artery shunting occurred in 14 (18.8%) patients, and after posterior tibial artery shunting - in 8 (11.6%) patients.

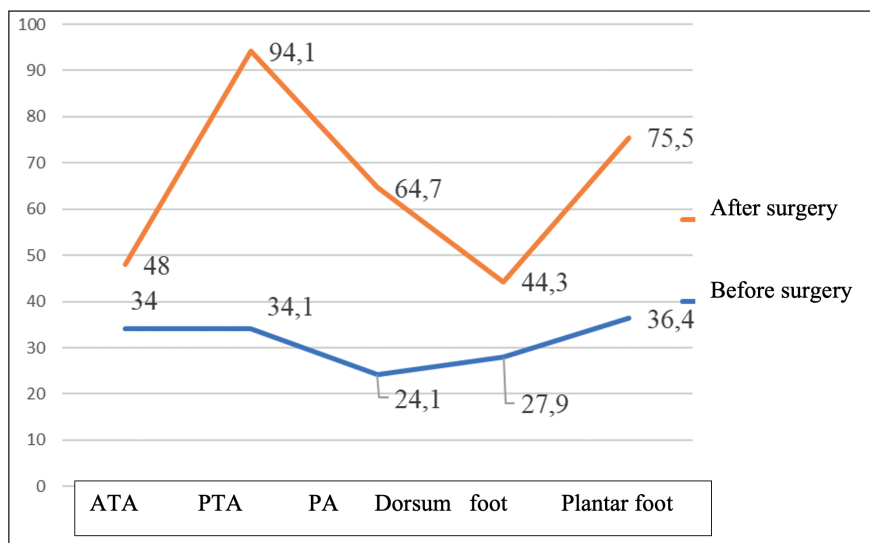
We did not observe thrombosis of the autovenous shunt in cause of using tibioperoneal trunk and peroneal artery as a tibia shunt artery.

Despite of a rather high percentage of unsatisfactory results of the direct methods of revascularization, the oxygen tension in the angiosomes of the leg and foot increased (table II).

According to the above data, a statistically significant difference was revealed in the regional perfusion index after surgery between the first and second groups in the angiosomal region of the posterior tibial artery and the peroneal artery ($p < 0.05$). When comparing other indicators, no statistically significant difference was found. Statistically significant differences were found in the groups ($p < 0.05$)

Table II. Index of regional perfusion of the leg and foot angiosomes after autovenous femoral-tibial shunting

Angiosoma	Surgery techniques			
	Distal autovenous femoral-tibial shunting		Distal autovenous femoral-tibial shunting + ROT	
	Before surgery	After surgery	Before surgery	After surgery
Anterior tibial artery	0,51±0,04	0,72±0,18	0,5±0,05	0,73±0,21
Posterior tibial artery	0,63±0,03	1,8±0,07	0,62±0,01	1,9±0,06
Peroneal artery	0,41±0,03	1,1±0,19	0,41±0,03	1,2±0,15
Dorsum foot	0,39±0,04	0,62±0,23	0,4±0,02	0,58±0,18
Plantar foot	0,4±0,04	0,83±0,23	0,41±0,03	0,85±0,26
t-test	$p < 0,05 (p = 0,037)$		$p < 0,05 (p = 0,046)$	

**Fig. 1.** Percentage ratio of tissue perfusion index relative to normal before and after surgery for distal autovenous femoral-tibial bypass grafting.

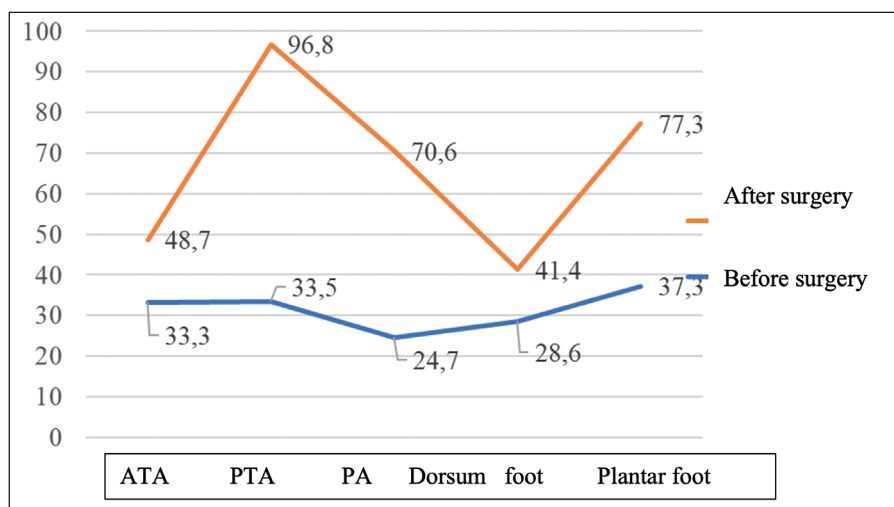


Fig. 2. Percentage ratio of tissue perfusion index relative to normal before and after surgery in cause of distal autovenous femoral-tibial bypass grafting in combination with ROT.

while comparing the indicators before and after surgery using the Student's t-test

At the same time, a stable increase in oxygen tension was observed in the angiosomes of the posterior tibial artery and / or peroneal artery in the case of the femoral-posterior tibial artery and bypass grafting of the femoral-tibioperoneal trunk; consistently low transcutaneous oxygen tension values were observed on the dorsum of the foot. In tibial arteries, the lowest regional perfusion index was observed in the anterior tibial artery basin below revascularization, despite of its usage as a receiving artery. (Fig. 1).

With the use of distal autovenous femoral-tibial bypass grafting, the indices of the regional tissue perfusion index in the angiosomes of the posterior tibial artery and peroneal artery increased almost threefold, and in the angiosomal basin of the dorsum of the foot and sole they doubled. The similar situation, even with better indicators was observed in patients who underwent distal autovenous femoral-tibial bypass grafting in combination with ROT. The indices in the angiosomes of the posterior tibial artery, peroneal artery increased threefold, the indices of regional perfusion in the angiosomes of the dorsum of the foot and sole doubled. (Fig. 2).

DISCUSSION

In recent years, due to the development of new technologies, the frequency of the lower extremities revascularization has increased significantly [9-14]. In cases where revascularization is not possible, the amputation percentage is 50% and increases over the next five years [7,10]. In the group of patients with successfully performed revascularization it ranges from 21.2 to 60% [1,2,3,7].

The main problem today is to find a way to stimulate the blood supply of the ischemic zone. One of the possible options is the restoration of blood flow in the maximum number of leg arteries; a number of other authors estimate that it is sufficient to restore blood flow only in anterior tibial artery and / or posterior tibial artery for a positive result; according to some data, only restoration of the vessel, which supplies blood to the affected area, can lead to a positive result of revascularization [1,3].

At the same time, when comparing the CT data, and the peculiarities of the trophic changes localization in the aspect of the angiosomal theory, most often trophic changes were found in the dorsal angiosome zone of the foot (52.4%), which comes from the anterior tibial artery. In 9 (42.9%) patients, trophic changes were found in the zone of the lateral plantar angiosome, which comes from the posterior tibial artery. In 4.8% of patients, necrosis was detected in the area of the medial-calcaneal angiosome, which comes from the posterior tibial artery at the border of the angiosome area from peroneal artery intermediate branch.

Angiosomal theory, despite a large amount of research, still raises many questions. This is primarily due to the lower extremities ischemia degree, as a treatment the treatment of foot ulcers in grade IV ischemia is a complex problem, that requires an integrated approach. A very important problem is the patients selection to the study groups for comparing the direct and indirect revascularization results. As a rule, indirect revascularization is performed only in cases of technical impossibility of direct revascularization, and this factor must be taken into account when evaluating the study results. Taking into account the fact that the "angiosomal artery" is most affected by the atherosclerotic process and the combined lesions of the foot arteries make up 30.4% of cases, only the skill of surgical revascularization can lead to the ischemia liquadation of the affected segment of the limb. After performing direct bypass surgery the peripheral blood flow and the volume of the microvasculature of the lower leg increase. In this case, performing indirect revascularization in addition will be more effective than usage this technique isolately for chronic critical ischemia treatment.

CONCLUSIONS

1. The patency of the femoral-tibial autovenous shunt during the year was 71%. High limb amputation was performed in 29% of patients.
2. According to CT data, the localization of trophic changes on the foot during critical ischemia of the lower extremities corresponds to the affected segment of the arterial angiosome, which supplies the corresponding area with blood.

3. After femoral-tibial autovenous bypass grafting, the highest levels of transcutaneous oxygen tension were observed in the basin of the posterior tibial artery and peroneal artery, and the lowest indicators of transcutaneous oxygen tension were observed in the basin of the anterior tibial artery.
4. During femoral-tibial autovenous shunting operations in combination with rotary osteotomies, the transcutaneous oxygen tension indices increased threefold in the angiosomal basin of the posterior tibial artery and peroneal artery, and twofold in the angiosomes of the dorsum of the foot and sole.

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ORCID and contributionship:

Mykhailo M. Lopit: 0000-0002-6425-2990^{B,C,D}

Vasily I. Rusin: 0000-0001-5688-9951^{A,F}

Patricia O. Boldizhar: 0000-0002-6295-5692^{B,E,F}

Fedir V. Gorlenko: 0000-0002-0496-2069^C

Olexander M. Kochmar: 0000-0003-4040-7561^C

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CORRESPONDING AUTHOR

Mykhailo M. Lopit

Uzhhorod National University

Str. Kapushanska 22, 88018 Uzhhorod, Ukraine

tel: +380671188100

e-mail: lopitmykhailo@gmail.com

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ORIGINAL ARTICLE

PATHOGENETIC ASPECTS OF METABOLIC SYNDROME IN EXPERIMENTAL ANIMALS

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Ihor Y. Dziubanovskiy¹, Andrii M. Prodan¹, Svitlana R. Pidruchna², Natalya A. Melnyk³, Volodymyr G. Dzhyvak⁴, Iryna M. Nikitina⁵

¹DEPARTMENT OF SURGERY FACULTY OF POSTGRADUATE EDUCATION I. YA. HORBACHEVSKY TERNIOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

²DEPARTMENT OF MEDICAL BIOCHEMISTRY I. YA. HORBACHEVSKY TERNIOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

³DEPARTMENT OF GENERAL HYGIENE AND ECOLOGY I. YA. HORBACHEVSKY TERNIOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

⁴DEPARTMENT OF CHILDREN'S DISEASES AND PEDIATRIC SURGERY I. YA. HORBACHEVSKY TERNIOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

⁵SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

The aim: Was to study the state of the nitric oxide system, LPO and antioxidant system in the body of experimental animals in simulated metabolic syndrome. The aim of the study was to study the state of the nitric oxide system, lipid peroxidation and antioxidant system in the body of experimental animals in simulated MS.

Materials and methods: The study was performed on 20 white male Wistar rats. Male control rats (n = 10) were fed a normal control diet. Male rats of the main group (n = 10) were fed a diet high in fat (over 60 % energy from fats) for 16 weeks, thus modeling the development of MS. The indicators of the prooxidant and antioxidant system, as well as the nitric oxide system were determined by photospectrographic method.

Results: In animals with simulated MS, intensification of lipoperoxidation (statistically significantly higher level of TBA-active products 1.84 times), depletion of antioxidant protection (statistically significantly lower level of superoxide dismutase 2 times), activation of nitric oxide system (statistically significantly higher NO-synthase level 2.15 times) were found compared with intact animals.

Conclusions: In animals with simulated MS, activation of lipid peroxidation processes, depletion of antioxidant protection and increased levels of nitrooxidative stress were found.

KEY WORDS: metabolic syndrome, peroxidation, free radicals, antioxidant protection, nitrooxidative stress

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INTRODUCTION

The prevalence of metabolic syndrome (MS), which includes a cluster of cardiovascular risk factors associated with obesity and insulin resistance, has recently increased dramatically and has become epidemic in many developed countries. This pathology is characterized by metabolic disorders such as hypertriglycerinemia, decreased levels of high-density lipoprotein, increased levels of low-density lipoprotein, insulin resistance, abnormal glucose tolerance and hypertension, which in combination with genetic predisposition to risk of type 2 diabetes, atherosclerosis and kidney, liver and heart disease [1].

MS is one of the most studied pathologies in the world due to the fact that such metabolic disorders are associated with common diseases of modern man - atherosclerosis, hypertension, type 2 diabetes, obesity. According to modern ideas, MS is characterized by a set of disorders of systemic regulation of lipid, carbohydrate, protein and other types of metabolism under the influence of external and internal factors. Separation of MS is of significant clinical importance, because on the one hand this condition is reversible, and on the other hand, it is necessary to address

the tactics of such a patient due to the fact that among people with MS risk of coronary heart disease or stroke 3 times higher, with a significant increase in mortality from cardiovascular disease [2]. The effectiveness of the use of different criteria for the detection of MS is unequal, which requires the necessary discussion and comparative analysis of existing diagnostic criteria and requires further in-depth study of biochemical parameters in MS.

One of the aspects associated with the development of MS and concomitant pathology is the excess of reactive oxygen species, which initiate peroxidation processes and cause damage to various cellular components. The accumulation of products of lipid and carbohydrate metabolism triggers detoxification reactions, including free radical processes [3]. Free radical processes are aimed at maintaining homeostasis, but at high intensity they can lead to the development of oxidative stress. Oxidative stress is one of the triggers that help to activate the body's cellular adaptation. The ratio of the activity of antioxidant systems and the amount of peroxidation products may vary depending on the state of the organism, the effects of various environmental factors. A normal stress response

may be accompanied by a short-term increase in reactive oxygen species. This is due to the reaction of adaptation of the organism in extreme conditions, in which reactive oxygen species play the role of secondary messengers, participating in the transmission of signal transduction, in the expression of a number of genes. The result is a timely mobilization of antioxidant protection, which reduces the level of reactive compounds, thereby preventing the manifestations of their toxic effects [4]. The toxic effect of reactive oxygen species is manifested in conditions of oxidative stress, which is accompanied by a sharp intensification of free radical processes and a decrease in the activity of antioxidant protection. Intensification of free radical processes and the development of oxidative stress is one of the pathogenetic links of many diseases, including cardiovascular, inflammatory, and aging. There are many works devoted to the study of free radical oxidation in various pathological conditions. However, data on the development of oxidative stress in MS is clearly insufficient, and this applies primarily to studies of the activity of antioxidant enzymes [5].

Another, no less important aspect of research in the pathogenesis of MS is the study of the role of mediators of intercellular interaction, which include nitric oxide and its metabolites. Nitric oxide is a universal regulator of various biochemical processes. Previously, the role of nitric oxide was associated only with inflammation, but it is involved in many physiological and pathophysiological reactions of the body, including pure apoptosis reactions. Nitric oxide and its metabolites are essential in the development of complications of MS. It is known that MS is accompanied by endothelial dysfunction, which is characterized by increased production of nitric oxide. Therefore, the increased attention of researchers is focused on this problem. Insufficient or excessive production of nitric oxide characterizes the presence of endothelial dysfunction, which is associated with a violation of the antioxidant system under the action of free radical oxidation [6]. This phenomenon is a major risk factor for the occurrence and complication of various diseases, including MS.

It is known that nitric oxide can cause both protective and damaging effects. It plays a significant role in the processes that regulate the production of free radicals. Its molecule itself as one of the reactive forms of oxygen is involved in the initiation of oxidative stress, which has independent antioxidant properties. However, the pathogenetic mechanisms that explain the role of nitrooxidative stress, lipid peroxidation (LPO) processes, the state of the antioxidant system in the development of MS and its complications have not been fully studied [8]. There are no clear criteria that would allow to have an idea of the course of this pathology, to allow to predict the course of the disease and to prevent undesirable consequences. Equally important is the search for effective, low-cost and prognostically successful methods of treating MS.

Thus, the role of nitric oxide activity, LPO processes, antioxidant enzymes in the pathogenesis of systemic disorders in MS remains unclear.

THE AIM

The aim of the study was to study the state of the nitric oxide system, LPO and antioxidant system in the body of experimental animals in simulated MS.

MATERIALS AND METHODS

The study was performed on 20 white male Wistar rats weighing 200–250 g (age 9–10 weeks), which were kept in standard vivarium conditions (air temperature: 22 ± 2 °C, humidity - 30-60 %, light / dark cycle: 12/12 hours). Male control rats ($n = 10$) were fed a normal control diet. Male rats of the main group ($n = 10$) were fed a diet high in fat (over 60 % energy from fat) for 16 weeks [9], thus modeling the development of MS.

At the end of the experiment, the animals were decontaminated by decapitation under thiopental anesthesia. The experiment complied with the requirements of the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986) and the European Union Directive 2010/10/63 EU on animal experiments. The Commission on Bioethics of Ternopil National Medical University named after I. Gorbachevsky (Prototol No. 12 of November 4, 2020) did not find any violations of moral and ethical norms during this study.

Determination of the content of TBA-active products (TBA-AP) was performed using the photospectrographic method. The principle of the method is the ability of secondary products of lipid peroxidation, namely malonic dialdehyde, when interacting with thiobarbituric acid (TBA) at high temperatures in an acidic environment to form a colored complex, the intensity of which is directly proportional to the content of TBA-active products (TBA-AP). Studies have been performed in and serum [10]. The content of TBK-AP was expressed in $\mu\text{mol} / \text{l}$ serum.

The level of *ceruloplasmin* (CP) was also determined by photospectrographic method [11]. Principle of the method: oxidation of p-phenylenediamine in the presence of ceruloplasmin leads to the formation of colored products. The amount of ceruloplasmin is proportional to the intensity of the color. The study was subjected to blood serum without traces of hemolysis. The result was expressed in mg / l .

The principle of the method [12] for determining the activity of *catalase* (CT) is based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate. The study was subjected to blood serum. Catalase activity was determined by photospectrometric method and expressed in mcat / l .

The principle of the method for determining the content of *reduced glutathione* (GSH) is that the interaction of 5,5'-dithiobis(2-nitrobenzoic acid (Elman's reagent) with free SN groups of reduced glutathione forms a thionitrophenyl anion, the amount of which is directly proportional to the group content of S [13]. The concentration of reduced glutathione in serum was expressed in mmol / l .

The *total antioxidant activity* of blood serum (TAA) was determined by photospectrographic method. The principle

Table 1. Indicators of prooxidant-antioxidant system in the serum of experimental rats ($M \pm m$)

Indicator	Groups of animals	
	Intact rats (n=10)	Rats with MS (n=10)
Blood plasma		
CP, mg / l	302,1 \pm 14,9	634,5 \pm 25,2*
CT, mcat / l	0,88 \pm 0,04	2,03 \pm 0,78*
GSH, mmol / l	3,90 \pm 0,29	2,03 \pm 0,18*
TAA, %	59,43 \pm 4,09	32,65 \pm 2,36*
TBA-AP, μ mol / l	8,50 \pm 0,49	15,76 \pm 1,09*
Liver		
SOD, units / g	0,77 \pm 0,04	0,36 \pm 0,03*

Note: * - statistically significant significance of the difference between indicators compared with the control group.

of the method is the ability of TAA to inhibit the formation of peroxidation products in the homogenate of the rat brain [14]. TAA was expressed in %.

Superoxide dismutase (SOD) activity was also determined by photospectrographic method [15]. Liver tissue homogenate was taken for the study. The activity of the enzyme was determined by its ability to inhibit the recovery of nitrotetrazolium blue. The percentage of inhibition was expressed in units / g.

The total content of *nitrites and nitrates* was determined by the Griss method after reduction of nitrates to nitrites with cadmium [16]. Calculations were performed according to the calibration schedule, using sodium nitrite as a standard. The content of nitrates and nitrites was expressed in mmol / l of blood serum.

The total activity of *NO-synthase* (NOS) in blood serum was determined colorimetrically by the amount of nitrates and nitrites formed in the incubation medium [17]. The

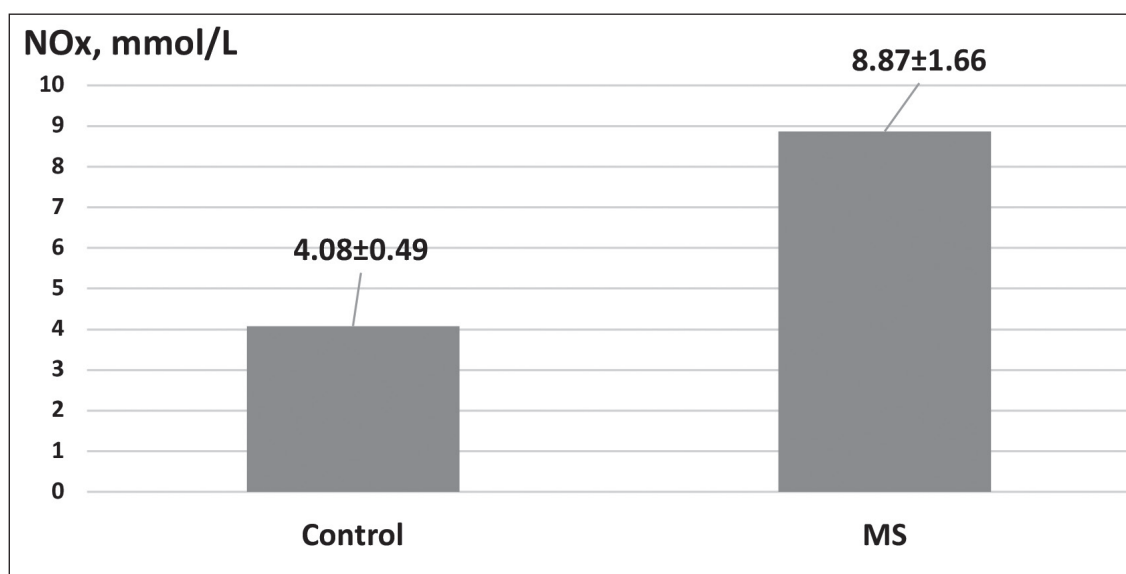
amount of nitrates and nitrites formed was determined as described below.

Statistical processing of the obtained research results was processed using the software Excel («Microsoft», USA) and Statistica.10.1. (Statsoft, USA), by the method of variation statistics using the Mann-Whitney U-test and the Student's test. Changes at $p < 0.05$ were considered statistically significant.

RESULTS

We have established a tendency to intensify lipoperoxidation processes and reduce the protective resources of antioxidant protection. Statistically significantly higher indicators of the content of TBA-AP in the serum of animals with simulated MS were established. As the results of our studies showed, the level of TBA-AP increased statistically significantly 1.84 times in animals with simulated MS (Table 1). There were also statistically significantly lower activity rates of SOD, TAA, and the level of SH-groups in the studied animals with simulated MS compared with intact animals. However, the content of CP and CT activity in our experiment increased. Thus, we found an increase in the content of CP in the blood plasma of animals with simulated MS in 2.1 times compared with the control group of animals. CT activity increased statistically significantly in the study group of animals by 2.3 times compared with the control. At the same time, in our experiment, the activity of SOD (2 times), TAA (1.8 times) and the content of GSH (1.9 times) decreased statistically significantly. This is apparently due to the depletion of the pool of antioxidant enzymes and the negative course of MS in experimental animals. The obtained data show that experimental MS contributes to oxidative and nitrooxidative stress, depletion of the antioxidant defense system.

When evaluating nitric oxide parameters, we recorded the development of endothelial dysfunction in rats with

**Fig. 1.** Concentration of nitrates and nitrites (NOx) in the serum of experimental rats

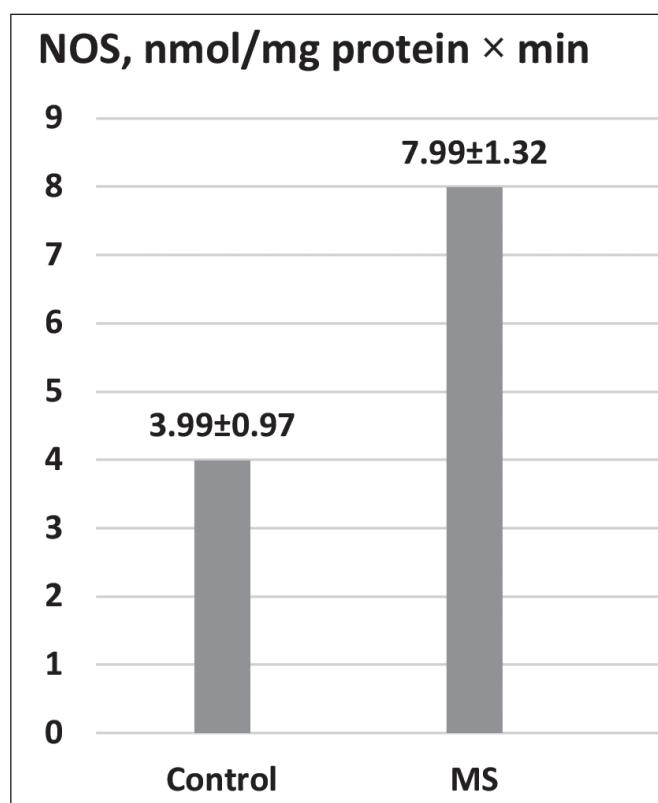


Fig. 2. Indicators of NO-synthase activity in the liver of experimental rats

simulated MS. There was a statistically significant increase in the concentration of nitrates and nitrites (NOx) in the serum of rats with experimental MS in 2 times compared with the control (Fig. 1).

We also found an increase in NO-synthase activity in the liver homogenate of animals by 2.15 times (Fig. 2). It is obvious that the increase in total NO-synthase activity registered by us is a consequence of activation of the inducible form of this enzyme, and the obtained fact of increase of nitrates and nitrites in blood serum of animals with the simulated metabolic syndrome is a consequence of excessive formation of nitric oxide and NOS, quickly converted to NOx.

Thus, the dynamics of the indicators established by us testifies to the development of nitrooxidative stress and imbalance in the prooxidant-antioxidant system in rats with simulated MS.

DISCUSSION

The obtained data show that experimental MS contributes to oxidative and nitrooxidative stress, depletion of the antioxidant defense system. We obtained data increasing the concentration of nitrates and nitrites (NOx) in the serum of rats by 2 times and increasing the activity of NO synthase in the liver homogenate of animals by 2.15 times with experimental MS compared with the control. It is obvious that the registered increase in the total activity of NO synthase is a consequence of activation of the inducible form of this enzyme, and our increase in nitrates

and nitrites in the serum of animals with simulated MS is a consequence of excessive formation of nitric oxide and NOS, which is unstable molecule and rapidly converted to NOx. At the same time, the products of partial oxygen reduction - superoxide-anion-radical - accumulate in the inflammatory focus [11-19]. In MS, high levels of NO in the body lead to interaction with the superoxide anion radical and the release of peroxynitrite anion, which in turn damages the vascular endothelium, which promotes the oxidation of lipids in cell membranes. In the presence of peroxynitrite or its breakdown products, thiol radicals of glutathione are formed, as a result of which the latter of the antioxidant is converted into a prooxidant, thereby initiating the processes of lipid peroxidation. In animals with experimental MS there is an increase in TBA-AP in 1.84 times.

Our data are consistent with the results of a number of studies, which found that in MS conditions there is increased activity of lipoperoxidation, and the oxidative stress that develops acts as an important pathogenetic mechanism of dysregulatory changes in metabolism. Changes in the prooxidant-antioxidant system under experimental MS can also contribute to the progression of metabolic disorders that are accompanied by the accumulation of lipids in cells [20]. Accordingly, free radical oxidation of lipids leads to increased levels of free fatty acids, triglycerides, cholesterol. It is known that the products formed in the intermediate stages of the peroxide cascade, in particular ketodienes and conjugated trienes, have a higher thermodynamic stability, as a result of which they are the initiators of numerous damaging effects at the level of biomembranes [21].

We can predict that the excessive accumulation of toxic products of lipoperoxidation obtained by us may further exacerbate existing damage, preceded by the appearance of more significant shifts in metabolism in MS. Overall antioxidant status is a limiting factor in the increased intensity of LPO. Activation of LPO processes leads to an imbalance in the antioxidant defense system, which deepens the development of complications in MS.

The most common in the literature data is the assessment of the intensity of free radical processes on the concentration of products of intermediate and final peroxidase catalysis: malonic dialdehyde, diene conjugates, ketodienes and trienes. The antioxidant system is studied by the activity of enzymes - SOD, CT, and indicators of non-enzymatic system of antioxidant protection - the content of GHS (SH-groups) [22].

According to the results of our own research, we have obtained data that are consistent with data from domestic and foreign studies. In particular, there is a tendency to intensify LPO processes and to reduce the protective resources of antioxidant protection. There were statistically significantly higher indicators of the content of TBA-AP, as well as statistically significantly lower indicators of activity of SOD, TAA, and the level of SH-groups in the studied animals with simulated MS compared to intact animals. However, the content of CP and CT activity in our experiment increased. Thus, we found an increase in the content of CP in the plasma of animals with

simulated MS in 2.1 times compared with the control group of animals, which may be due to the fact that the specific SOD activity of ceruloplasmin in rats is associated with an additional site for copper ion binding in this enzymes [23–24]. Since CP is to some extent able to inhibit the respiratory explosion of neutrophils due to SOD activity, it can be assumed that this mechanism of protection against oxidative stress associated with inflammation in animals with MS is more pronounced.

Also in our study, we found an increase in catalase activity in animals with MS in 2, 3 times compared with the control group of animals. The increase in the activity of the studied enzyme is associated with the inclusion of compensatory mechanisms of the antioxidant defense system. However, in our experiment, the activity of SOD (2 times), TAA (1.8 times) and the content of GHS (1.9 times) was statistically significantly reduced, which is obviously associated with the depletion of the pool of antioxidant enzymes and negative course of MS in experimental animals.

Under the conditions of our experiment, the level of free radical oxidation and inhibition of antioxidant complexes increased, which leads to the accumulation of free radicals in the serum of animals with simulated MS. In general, the presence of inflammation, hypoxia and oxidative stress enhances the synthesis of cytokines that express inducible NO-synthase, and the latter generates high levels of NO production. Changes in the processes of LPO, the state of antioxidants, the intensity of nitric oxide formation cause the development of MS [25–26]. Therefore, the question of studying the intensity of the course of pathological free radical oxidation, which causes a violation of the integrity of the vascular endothelium in MS, remains relevant. Studies confirm the fact that the activity of free radical reactions contributes to severe vascular endothelial cell dysfunction. In turn, it plays a leading role in the violation of vascular tone and the development of atherosclerotic lesions of the arteries. In this regard, the endothelium, its functions and correction of their disorders require new searches for their correction and prevention of complications of MS [27–28]. Thus, the results indicate the important role of nitrooxidative stress, activation of lipoperoxidation processes, disturbances in the system of pro- and antioxidant protection in the formation and progression of MS and the need to seek its surgical correction.

CONCLUSIONS

In animals with simulated metabolic syndrome, activation of lipid peroxidation processes (at a statistically significantly higher level of TBA-AP), depletion of antioxidant protection (at a statistically significantly lower level of SOD) and an increase in nitrooxidative stress (at a statistically significantly higher level of nitric oxide) were found. These disorders are due to the deepening of metabolic imbalance in the development of metabolic syndrome.

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ORCID and contributionship:

Ihor Y. Dziubanovskyi: 0000-0001-8852-3938 ^{A, D, E, F}
 Andrii M. Prodan: 0000-0002-6052-192X ^{A, B, D, F}
 Svitlana R. Pidruchna: 0000-0002-5607-568 ^{A, E}
 Natalya A. Melnyk: 0000-0002-7357-7551 ^{B, D}
 Volodymyr G. Dzhyvak: 0000-0002-4885-7586 ^{B, C}
 Iryna M. Nikitina: 0000- 0001-6595-2502 ^{E, F}

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CORRESPONDING AUTHOR

Andrii M. Prodan

I. Horbachevsky Ternopil National Medical University
 Maidan Voli str., 1, 46001 Ternopil, Ukraine
 tel: +380677120199
 e-mail: prodan@tdmu.edu.ua

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ORIGINAL ARTICLE

COGNITIVE AND STRUCTURAL CHARACTERISTICS OF LATIN TERMS FOR INFECTIOUS AND INVASIVE DISEASES WITH A ZOOMORPHIC COMPONENT

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Olena M. Bieliaieva¹, Valentyna H. Synytsia², Olena V. Myronyk², Yuliia V. Lysanets¹, Iryna M. Solohor¹, Halyna Yu. Morokhovets¹, Svitlana M. Efendiieva¹

¹POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

²BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

The aim was to analyze the morphological, structural and lexico-semantic presentation of the Latin terms denoting the infectious and parasitic diseases, part of which is a zoonymic component.

Materials and methods: The presentation sample was made by the method of continuous sampling based on textbooks, manuals, including the three-volume edition "Infectious and parasitic diseases", and a number of modern dictionaries. Structural, semantic and descriptive methods were used to address the aim of the research.

Results: Monolexic composites with a zoomorphic component are formed in a suffixal way. In multi-word phrases, syntactic constructions of mixed type, are usually used and they are characterized by the combination of words of Greek and Latin origin, but these formations are few. The eponymous and toponymic formations to denote specific nosological forms in helminthology are not typical. The data on the first contribution of a researcher to the description of the disease in the name of the term is not displayed, preference is still given to the name of the pathogen, rather than the name of the researcher. The prefix-suffix-based method of formation of terms denoting infectious and parasitic diseases with a zoonymic component is observed sporadically.

Conclusions: The comparison of the above-analyzed terms with the corresponding terminological units of the English medical terminology indicates the predominant use of Latin terminological units. In contrast to many clinical terms with a zoomorphic component, where certain associative moments are taken into account.

KEY WORDS: medical terminology, Latin terms, infectious and invasive diseases, a zoomorphic component, cognitive and structural approach

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INTRODUCTION

Acquaintance with scientific publications of the last ten years testifies to the interest of researchers in terms of various sublanguages of medicine, in which a zoomorphic component is traced, although the number of studies in this direction is insignificant. Perhaps the greatest attention is paid to the coverage of zoomorphic components based on the material of Latin anatomical terms (Y. Brazhuk, 2014; O. Kharik, 2017); features of metaphorical connotative meanings of medical terms with an animal component in modern Chinese researches (K. Cui, 2014). O. Kryzhko's publications (2010, 2015) are devoted to the cognitive features of zoonymic terms of the Ukrainian language in semasiological and onomasiological aspects; 97 lexical and semantic units with zoonymic and ornithonymic components based on the material of Russian and English medical vocabulary were analyzed by A. Ryabova and O. Kozlova (2012); some remarks on the functioning of terms with an animal component in the "Infectology" terminology are set out in the research by V. Synytsia (2019). However, we have not found any studies that would comprehensively focus on the structural and semantic aspects of the nomination

of Latin terms with the use of zoonyms as monolexic and terminological phrases of the microfield "infectious and invasive diseases". The choice of this area of research is also explained by the extreme prevalence of diseases caused by pathogenic protozoa, helminths or arthropods among adults and children. The number of terms with an animal component is quite numerous and requires a detailed study, which will ensure an in-depth understanding of the functioning of nominative units from the outlined microfield of medicine from the standpoint of cognitive and structural approach.

THE AIM

The aim of the research is to analyze the morphological, structural and lexico-semantic presentation of the Latin terms denoting the infectious and parasitic diseases, part of which is a zoonymic component.

MATERIALS AND METHODS

The presentation sample was made by the method of continuous sampling based on textbooks, manuals, including

the three-volume edition “Infectious and parasitic diseases”, and a number of modern dictionaries [1–8]. Structural, semantic and descriptive methods were used to address the aim of the research.

RESULTS AND DISCUSSION

The largest number of terms with a zoomorphic component is found in medical helminthology, a section of medical parasitology that studies worms and the diseases they cause. The term “helminthologia” itself refers to one-word two-morpheme composites: the first position is occupied by the term element “helminth-“, which comes from the noun of Greek origin ἕλμινς, ἰνθος – “worm” (Latin version *helmins*, *helminthis*, base *helminth-*) and the final term element “-logia”. The lexeme *helmins*, *inthis f* has come down to this day due to the surviving works of Hippocrates, and in modern dictionaries it is interpreted as “a group of parasitic worms, which includes representatives of the classes of trematodes, cestodes, nematodes; pathogens of helminthic diseases of humans and animals” [8, p. 392]. The concept of “helminthiasis / helminthiasis: a disease caused by helminths; worm infestation” is codified by the Latin terms *helminthosis* (base *helminth-* + suffix *-osis*), *helminthiasis* (base *helminth-* + suffix *-iasis*) and *helminthismus* (base *helminth-* + suffix *-ismus*). Doctors in most countries of the world attribute helminthiasis to both parasitic and infectious diseases. Focusing on the ways of transmission, the famous Soviet helminthologist, Academician K.I. Skriabin (1878–1972) and Professor R.S. Schultz (1896–1973) identified two epidemiological groups of helminthiasis and introduced the concepts “biohelminthosis” and “geohelminthosis” into medical discourse in 1937. The terms “biohelminthosis” and “geohelminthosis” refer to one-word three-component formations: *biohelminthosis* ← bi- (from the Greek noun βίος – *life*) + *helminth-* (from the Greek noun ἕλμινς – *worm*) + Greek suffix *-osis* – *invaders*, whose pathogens develop and are transmitted to humans through the tissues of their body [2, p. 254]; *geohelminthosis* ← ge- (from the Greek noun γῆ – *earth*) + *helminth-* (from the Greek noun ἕλμινς – *worm*) + Greek suffix *-osis* – *invasions*, whose causative agents develop directly (without the participation of an intermediate host) and are transmitted to humans through the elements of the environment, contaminated with invasive eggs or larvae [2, p. 254]. In 1952, Professor E.S. Shulman identified an additional group – contact helminthiasis. To denote this concept, medical dictionaries offer a two-word term “helminthosis contactilis”, which is etymologically of Greek-Latin origin: single-word composite of Greek origin *helminthosis* + Latin adjective *contactilis*, *e*, formed by the suffix “-al” from the past participle, passive *contactus*, *a*, *um* of the verb *contingo*, *tigi*, *tactum*, *ere* – “to touch”. The article by M. Teleky elaborates on the terminological units with an adjective component in the medical terminology system in more detail [9], where, in particular, the suffix “-al” is mentioned, among others, as one that belongs to a small group of deverbal adjectives, which are found in medical

terminological phrases. However, analyzing the terminological phrases of infectious and invasive diseases with a zoomorphic component, we can question the above statement, because we found six phrases with adjectives, which used the suffix “-al”: *myiasis intestinalis*, *myiasis nasalis*, *helminthosis naturalis et focalis*, *schistomiasis intestinalis*, *schistomiasis urogenitalis*, whereas only 4 terminological formations with the suffix “-os” were revealed: *myiasis mucosa*, *myiasis dermatosa*, *myiasis iomaginosa*, *myiasis oestruesa*; and with the suffix “-ic” – only two examples: *myiasis traumatica*, *ascaridosis hepatopancreatica*.

According to the unified nomenclature of invasive diseases, the dominant structural model is schematically represented as follows: the Greek suffix “-osis / -iasis” is added to the generic name of the parasite. For example, the term “worm disease caused by *Ancylostoma duodenale* or *Necator americanus*” is verbally denoted by the term *ankylostomosis* or its variant *ankylostomiasis* [7, p. 118]. Given the nomenclature of parasitic diseases, this nomination is structurally a two-component formation: the first position is occupied by the generic name of the pathogen *Ankylostoma* (or rather, the truncated form of *ankylostom-*), which is joined by the suffix “-osis” (or as a variant “-iasis”). However, one must not forget that the generic name of the pathogen *Ankylostoma* is etymologically derived from the Greek word ἀγκύλος – *curve* + Greek noun στόμα – *mouth* and is also a two-component formation. That is, if we “decompose” the term *ankylostomosis* / *ankylostomiasis* without taking into account the name of the pathogen, we can distinguish three morpheme components: σκλαγκύλος + στόμα + “-osis / -iasis”.

The study found that the dictionaries by Arnaudov [6, p. 23] and Rudzitis [7, p. 118] record spelling forms *Ankylostoma*, *ankylostomosis* / *ankylostomiasis*, whereas the encyclopedic dictionaries by Dornald [5, p. 100] and Petrukh [8, p. 143] – *Ancylostoma*, *ancylostomosis* / *ancylostomiasis*. Moreover, in the dictionary by Dornald [5] on p. XX, which lists the “The list of analytical words”, two initial terminological elements are given without any explanation: “ancyl-” and “ankyl-”. In our opinion, the variant “ancyl-” contradicts the rules of Latinization of the Greek words, because to transmit the sound [k], available in the Greek word ἀγκύλος, one must use the letter of Greek origin [k], because according to the phonetics of Latin, the letter [c] in the position before [y] is pronounced [ts]. It should also be remembered that the combination of the Greek letters γκ corresponds in Latin to the combination “nk”, not “nc” [6, p. XVIII], which again indicates that the spelling of the above terms is not entirely correct. However, a review of recent scientific papers published on the Internet shows that the variant *Ancylostoma*, *ancylostomosis* / *ancylostomiasis* can be found more often in medical discourse. It is thought that one reason may be the influence of English, another – the reluctance of the medical community to take into account the etymology of a terminological unit due to lack of knowledge of the phonetic system of the language from which the term was borrowed, in this case – the ancient Greek.

In ICD-10, code B-76.0, the invasion, caused by *Ankylostoma* species is referred to as “ankylostomiasis/ankylostomosis”, whereas the common name for code B-76 is “ankylostomidosis (hookworm disease)”. Thus, the term “hookworm disease” refers to a disease that combines two invasions: ankylostomosis / ankylostomiasis, caused by *Ankylostoma duodenale*, and necatorosis, caused by *Ankylostoma americanum*, also known as *Necator americanus*. The term “ankylostomidosis” is formed according to a well-known model: the Greek suffix “-osis” is added to the name of the family of nematodes of the class Rhabditida *Ankylostomatidae*: *ankylostomidosis*.

In general, in ICD-10, subheading B65 - B99 “Helminthiasis” of heading A₀₀ - B₉₉ Class I. “Some infectious and parasitic diseases” record 19 nosologies, where a zoomorphic component is distinguished. Quantitative analysis of the selected terminological units is as follows: two two-component formations with the suffix “-osis”, five – with the suffix “-iasis” and several word-forming variant forms, which have a common root, equivalent lexical and grammatical meaning, but the discrepancy is in the use of close by semantic load, but different in the verbal expression of suffixes: *helminthosis et helminthiasis*, *taeniosis et taeniasis*, *trichinosis et trichiniasis*, *bilhaziosis et bilhaziasis*; there are nine three-component one-word nominations; among them, eight are formed with the suffix “-osis” and one variant form: *ankylostomosis et ankylostomiasis*. The four-component terms are represented by three composites: word-forming variant forms *diphyllobothriosis et diphyllobothriasis* and lexemes *trichostrongyloidosis* and *angiostrongyloidosis*.

Regarding the morphological nature of term components, the following conclusion can be made:

1) noun morpheme of Greek origin + suffix “-osis” or “-iasis” – these are the most numerous examples. Let us illustrate this statement with several examples: *ascaridosis* ← basis of “ascarid-” from the Greek noun ἄσκαρις – “worm”, + suffix “-osis”. It is known that the term “ascarid” (*Ascaris, idis f*) was introduced by Hippocrates, and the modern name *Ascaris lumbricoides* – a roundworm of the nematode class – was given to the helminth by Carl Linnaeus in 1758.

2) two noun morphemes of Greek origin + suffix “-osis” or “-iasis”: *echinococcosis* ← base *echin-* from the Greek noun ἐχῖνος - *hedgehog* + Greek noun κόκκος - *grain* + suffix “-osis”; *cysticercosis* ← base *cyst-* from the Greek noun κύστις - *bubble* + Greek noun κέρκος - *tail* + suffix “-osis”; *trichocephalosis* ← basis *trich-* from the Greek noun θρίξ - *hair* + Greek noun κεφαλή - *head* + suffix “-osis”, etc.

3) prefix of the Greek origin + adjective morpheme + suffix “-osis” / “-iasis” – three examples: *metastrangylosis* ← Greek prefix *meta* + base *strongyl-* from the Greek adjective στρογγύλος - *round* + suffix “-osis”; *metagonimosis* / *metagonimiasis*: ← Greek prefix *μετα* + base *gonim-* from the Greek adjective γόνιμος - *fruitful* + suffix “-osis” / “-iasis” and *paragonimosis* / *paragonimiasis*: ← Greek prefix *παρα* + base *gonim-* from the Greek adjective γόνιμος - *fruitful* + suffix “-iasis”.

Medical terminology in general is characterized by the use of a huge number of terms, where one of the components refers to the name of a certain person, to whom we owe the functioning of a particular eponymous term. This phenomenon is inherent in the terminology of all subsystems of medicine, as evidenced, for example, by the publications on eponymous terms based on the material of English and Latin terminology [9; 12 - 17]. However, we did not find any mention of eponymous formations in the names of helminthiasis, therefore, we also paid attention to this aspect. The analysis of verbal notations of the concept in the above-mentioned thematic field revealed the existence of several terms, which can be attributed to eponymous ones. In particular, it is a one-word composite *brugiosis* - helminthiasis from the group of filariasis caused by *Brugia malayi*. Thus, the term is based on the name of the Dutch parasitologist S.L. Brug (1879-1946), who isolated a helminth called *Filaria malayi* in 1927. However, in 1958 (according to other sources, in 1960), D. Buckley proposed to classify the pathogen in the genus *Brugia* - a genus of filamentous worms of the superfamily Filarioidea, which parasitize in humans and mammals, a new species named *Brugia malayi* in honor of the discoverer S.L. Brug, to which the medical community of the time responded favorably.

Another monolexemic composite is represented by the term *bilharziasis* / *bilharziosis*, which corresponds to the name of the German doctor Th.M. Bilharzar (1825-1862), who in 1851 described the genus of trematodes *Bilharzia*, parasitizing in blood vessels, and after whom this genus was named.

Another name for helminthiasis *wuchereriosis* / *wuchereriosis* has its origins in the name of the German physician Otto Wucherer (1820-1873), to whom we owe the existence of the name *Wuchereria* of the genus *Nematode* of the superfamily Filarioidea.

Eponymous terminological phrases include *schistosomiasis* / *schistosomiasis* *Mansoni* - infection with trematodes of the species *Schistosoma mansoni*, which live mainly in the superior and inferior mesenteric veins. As you observe, the general term *schistosomiasis* (from the Greek adjective σχιστός - *split* Greek noun σώμα - *body* + Greek suffix “-osis”) is used to verbalize the concept, which means “helminthiasis caused by trematodes of the genus *Schistosoma*” and the name of the English doctor Sir Patrick Manson (1844-1922), who in 1898 proposed to distinguish between two forms of schistosomiasis: the intestinal (*schistosomiasis intestinalis*) and the urogenital ones (*schistosomiasis urogenitalis*). Later, in 1907, the Italian and English physician Louis Western Sambon (1867-1931) suggested that intestinal schistosomiasis should be named after the person who first noticed helminth eggs in a patient's feces in South America, namely *schistosomiasis* *Mansoni* [10]. Modern encyclopedic medical dictionaries record two synonymous names for the transmission of tropical helminthiasis, which occurs with allergic manifestations and signs of damage to the mucous membrane of the colon by migrating eggs of the helminth *Schistosoma mansoni*: *schistosomiasis* *Mansoni* and *intestinal beniponia*. These terminological units are absolute synonyms, and their parallel functioning

in medical discourse is explained by the historical stages of the study of helminthiasis caused by the genus of trematodes of the family Schistosomatidae. Quite correct in this regard is the remark of M. Kuzmin that one scientific concept can have many different features, it can be perceived differently and have several names that reflect the different properties of the object of nomination [11, p. 78].

In addition to the eponymous formation recorded in the dictionary article “schistosomiasis”, we should pay attention to the two-lexemic composite with a toponymic component. It is a terminological phrase *schistosomiasis japonica* - helminthiasis of the group of trematodes, which is caused by the species *Schistosoma japonicum* and is characterized mainly by lesions of the gastrointestinal tract, where the term *schistosomiasis* occupies a constant position, and the variable part is expressed by the adjective. Since the parasite was first discovered in 1904 by the Japanese parasitologist Fujiro Katsurada (1867-1947) in a patient from Kofu, Yamanashi Prefecture (Japan), the genus *Schistosoma* was joined to the species *japonicum*, which fixed the name of the area where the helminth eggs were first isolated.

The term “loaosis” refers to “hybrid” terminological units, as the first position is not occupied by the classical (Greek or Latin) component, but by “the local word in Angola, West Africa” [5, p. 1328], *loa* – “the eye worm” [3, p. 310]. In 1778, the French surgeon F. Guyot described the parasite and gave it the name *Loa loa*, and the disease became known as *loaosis*.

The vast majority of the studied names of helminthiasis are characterized by terminological compatibility, which seems to us a well-motivated phenomenon: a specific pathogen that causes a specific disease, and the suffix “-osis” / “-iasis”. For example, the larvae of nematodes *Toxocara canis* or *Toxocara mystax* cause severe parasitic toxocarosis; the Chinese sucker *Clonorchis sinensis* is the causative agent of chronic helminthiasis, known as clonorchosis; despite the two species of *Fasciola hepatica* et *Fasciola gigantica*, helminthiasis is codified by one term – “fasciolosis”.

However, parasitism of four types of schistosomes in the human body has led to the emergence of terminological phrases, which differ depending on the predominant helminthic damage of the organ. Thus, *schistosomiasis urogenitalis* informs about the predominant lesion of the urogenital organs (*urogenitalis*, e); *schistosomiasis intestinalis* indicates intestinal invasion (*intestinalis*, e); *schistosomiasis intercalatum* is caused by *Schistosoma intercalatum* and is an endemic intestinal disease prevalent in West Central Africa; *schistosomiasis japonica* is characterized by damage to the digestive system and its spread in the regions of southern Japan, southern China, and the Philippine archipelago.

CONCLUSIONS

The conducted study allows us to draw the following conclusions:

1) monolexemic composites with a zoomorphic component are formed in a suffixal way: the Greek suffix “-osis” / “-iasis” is added to the name of the genus of the pathogen,

which occupies the initial position; the vast majority of terms are of Greek origin;

2) in multi-word phrases, syntactic constructions of mixed type, are usually used and they are characterized by the combination of words of Greek and Latin origin, but these formations are few;

3) eponymous and toponymic formations to denote specific nosological forms in helminthology are not typical. The data on the first contribution of a researcher to the description of the disease in the name of the term is not displayed, preference is still given to the name of the pathogen, rather than the name of the researcher. For example, chronic helminthiasis with a predominant lesion of the small intestine was first described by the French physician L. Norman in 1876 under the name “Cochin-China diarrhea”, because signs of the disease were found in soldiers returning from Cochin-China, one of the districts of the Mekong River Delta in southeastern Indochina. However, none of the dictionaries we relied on in the study recorded terms related to the doctor’s name or geographical area. Three years later, in 1879, the eminent Italian parasitologist Giovanni Battista Grassi (1854-1925) provided in-depth information on the parasite’s life cycle and proposed a name for a new genus of *Stoangyloides* parasites, resulting in the disease now known as *strongyloidosis* / *strongyloidiasis*;

4) prefix-suffix-based method of formation of terms denoting infectious and parasitic diseases with a zoonymic component is observed sporadically;

5) comparison of the above-analyzed terms with the corresponding terminological units of the English medical terminology indicates the predominant use of Latin terminological units, cf.: *schistosomiasis* (Latin and English); *strongyloidosis* (Latin and English) etc., which once again confirms the influence of Latin on the formation of the English medical terminology;

6) in contrast to many clinical terms with a zoomorphic component, where certain associative moments are taken into account, cf.: *auris felina* - cat’s ear (because of similarity in shape), terms with an animal component in infectology emphasize the name of the pathogen: *onchocercosis* – from the name of the pathogen - round helminth *Onchocerca volvulus* (etymologically the generic name is derived from the Greek noun *onkos* – “tumor” and the Greek noun *kerkos* – “tail”).

PROSPECTS FOR FURTHER RESEARCH

In our opinion, it is quite logical to carry out a comparative analysis of the terms of infectious and invasive diseases with a zoomorphic component based on the example of modern European languages, focusing on the current International Classification of Diseases, revision 10, to draw relevant conclusions about derivational characteristics of terms denoting a group of intestinal diseases caused by different types of pathogens. Our previous investigation, in particular, of the verbal codification of the concept of “a disease caused by *Ascaris lumbricoides*” confirmed the conclusion about the influence of Latin on the formation of national terms of infectious and invasive diseases with a zoomorphic component (Table I):

Table I. The verbal codification of the concept of “a disease caused by *Ascaris lumbricoides*”

Language	Term
Latin	ascaridosis, ascaridiasis, ascarisiasis
English	ascaridiasis, ascariasis
German	Askariasis, Ascaridiasis
French	ascaridiase, ascaridiose
Italian	ascaridiasi
Polish	ascaridoza
Ukrainian	аскаридоз
Russian	аскаридоз

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ORCID and contributionship:

Olena M. Bieliaieva: 0000-0001-9060-4753^{A,D,F}
Valentyna H. Synytsia: 0000-0002-8346-3662^{E,F}
Olena V. Myronyk: 0000-0002-5717-7267^{B,D}
Yuliia V. Lysanets: 0000-0003-0421-6362^{B,D}
Iryna M. Solohor: 0000-0001-9899-6552^B
Halyna Yu. Morokhovets: 0000-0002-6079-6878^B
Svitlana M. Efendiieva: 0000-0002-9633-0236^B

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CORRESPONDING AUTHOR

Yuliia V. Lysanets
Poltava State Medical University
23 Shevchenko Str., 36011 Poltava, Ukraine
e-mail: julian.rivage@gmail.com

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ORIGINAL ARTICLE

ECOLOGICAL EDUCATION AND ITS RELATIONSHIP WITH STUDENTS' HEALTH

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Grygoriy P. Griban¹, Ivan M. Okhrimenko², Mykhailo S. Myroshnychenko³, Oleksandr A. Tomenko⁴, Serhii O. Matrosov⁴, Mariia M. Rohovenko²

¹ZHYTOMYR IVAN FRANKO STATE UNIVERSITY, ZHYTOMYR, UKRAINE

²NATIONAL ACADEMY OF INTERNAL AFFAIRS, KYIV, UKRAINE

³KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

⁴SUMY STATE PEDAGOGICAL UNIVERSITY NAMED AFTER A.S. MAKARENKO, SUMY, UKRAINE

ABSTRACT

The aim is to assess the state of ecological education in student youth and determine its relationship to the level of students' health.

Materials and methods: The research was conducted on the basis of the Polissya National University (Zhytomyr, Ukraine) and the Sumy State Pedagogical University named after A.S. Makarenko (Sumy, Ukraine) in 2018–2020. The ascertaining experiment, which was conducted to assess the state of ecological education of students according to the questionnaire developed by us involved 503 students. The formative experiment aimed at studying the relationship between the level of ecological education and the level of students' health involved 59 students.

Results: The ascertaining stage of the experiment showed that the vast majority of students of different specialties revealed a low level of mastery of the components of ecological education. The formative stage of the experiment showed that the EG students significantly improved the level of all components of ecological education, as well as the level of their health ($p < 0.001$). No significant changes were revealed in the CG ($p > 0.05$).

Conclusions: The research showed that purposeful work on the formation of ecological education in the process of fitness and health recreation activities has a positive effect on all its components (knowledge, character building, worldview, culture) and the level of students' health. In general, it will help to improve the efficiency of the educational process of students, improve indicators of their life-sustaining and future professional activities.

KEY WORDS: ecological education, environmental factors, physical education, health, students

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INTRODUCTION

Today, students face a difficult task that consists in making radical changes in their consciousness and behaviour, forming and voluntarily accepting the restrictions and prohibitions dictated by the laws of nature. Fundamental changes are also needed in the education system, its values and ways of life, taking into account the environmental, economic, political, social and spiritual needs of today. Therefore, one of the ways to improve the efficiency of the educational process in physical education, the quality of sporting and mass participation as well as fitness and health recreation events including maintaining the health of student youth is to involve the issues of environmental education in the curriculum of physical education in higher educational institutions (HEI) of Ukraine, which should provide students with a system of knowledge about the positive and negative impact of environmental factors on human life and activities; to provide recommendations for exercise and sports in a polluted environment, including various temperature factors, polluted air, water, food, etc.

Excessive technological load on the environment; too slow implementation of waste-free processes; the lack of a unified environmental policy has led to the fact that much of Ukraine is in a zone of environmental disaster. This, in turn, has worsened human health, reduced life expectancy and fertility, increased the risk of poisoning from the polluted environment and the probability of consuming unsafe food i. e. the problems caused by technology-related changes in the transformation of the biosphere. These problems are called environmental, the responsibility of solving which is now laid upon the state [1, 2, 3].

Individual elements of the environment that interact with the human body are called environmental factors. Any environmental factor can be perceived by the human body positively or negatively, depending on the dose of exposure. Each person has his own optimal dose of a factor [4, 5]. The life-sustaining activities of the human body is in a continuous dynamic relationship with environmental factors. This interaction should not disrupt the adaptive mechanisms of the human body. Unconditional and conditioned reflexes are created in the human body

under the influence of various stimuli of his internal and external environment, which determine the maintenance of dynamic balance based on the exchange of substances and energy between the body and the environment [6, 7].

Environmental factors must have a positive effect on health and ensure the normal course of all human life processes. Environmental factors are divided into three groups according to their nature: 1) abiotic ones stipulated by the influence of inanimate nature (temperature, solar radiation, light, water, wind, precipitation, relief, etc.); 2) biotic ones i. e. the influences of wildlife and all relationships with it; 3) anthropogenic ones i. e. the influences caused by human activities [8, 9].

Environmental factors have a very complex physico-chemical composition, including temperature, pressure, humidity, air movement, electric and magnetic field, radiant energy, chemicals released into the air by plants, and so on. Environmental factors affect all receptors and the body as a whole due to such a complex structure. The vastness and generalization of their action is explained by the involvement of various levels of structural and functional organization in the reaction i. e. from molecular and cellular to organ and organism related ones, from peripheral nerve centres to the psycho-emotional sphere of a human being [10, 11].

The above gives grounds to ascertain that the effectiveness of educational and professional activities and in general the life of students should be based on the perception and analysis of information about the characteristics of the environment in which these activities takes place.

THE AIM

The aim is to assess the state of ecological education in student youth and determine its relationship to the level of students' health.

MATERIALS AND METHODS

Research participants. The research was conducted on the basis of the Polissya National University (PNU, Zhytomyr, Ukraine) and the Sumy State Pedagogical University named after A.S. Makarenko (SSPU, Sumy, Ukraine) in 2018-2020. The ascertaining experiment, which was conducted in 2018, involved 503 third and fourth year students of these higher educational institutions from different faculties: law, public administration and national security; economics and management; engineering and energy; forestry and ecology; accounting and auditing; veterinary medicine as well as agronomic and technological one and other faculties in various specialties. Assessment of the state of ecological education of students was carried out according to the questionnaire developed by us (Appendix I), which contained questions in four blocks: ecological knowledge (6 questions), ecological character building (3 questions), ecological worldview (3 questions), and ecological culture (4 questions).

A questionnaire created by the authors of this article was used for the survey in accordance with the requirements of

the Codes of Ethics of PNU and SSPU. The questionnaire contains 16 questions and is aimed at studying of the state of ecological education of student youth. The questionnaire was assessed by the experts in this field (8 professors and 11 associate professors) and was approved by the Academic Council of PNU (Protocol No. 12 dated 18.08.2018) and SSPU (Protocol No. 12 dated 29.08.2018). Consent to voluntary participation in the survey was obtained from all the students involved in the study.

The completeness of the disclosure of the issue, integrity, logical sequence and knowledge were taken into account when performing written tasks. The results were assessed on the unified 100-point scale according to the current system of comprehensive assessment of students' knowledge in universities, in order to stimulate planned and systematic educational work. 90-100 points – the student shows personal creative abilities, is able to acquire knowledge independently, finds and processes the necessary information without the help of the teacher, is able to use the acquired knowledge and skills to make decisions in unusual situations, convincingly argues answers, independently reveals his own talents and inclinations. 82-89 – the student has excellent skills in the studied amount of material, applies it in practice, freely solves exercises and problems in standard situations, and independently corrects mistakes the number of which is insignificant. 75-81 – the student is able to compare, summarize, systematize information under the guidance of the teacher; as a whole is able to apply it independently in practice; to control his own activities; to correct mistakes that include significant ones, to choose arguments to confirm opinions. 64-74 – the student reproduces a significant part of the theoretical material, shows knowledge and understanding of the basic provisions; can analyse the educational material with the help of the teacher, correct mistakes that include a substantial number of significant ones. 60-63 – the student masters the educational material at a level higher than the elementary one, a significant part of it is reproduced at the reproductive level. 35-59 – the student masters the material at the level of individual fragments that make up a small part of the study material. 1-34 – the student masters the material at the level of elementary recognition and reproduction of individual facts, elements and objects.

The experimental ($n = 28$) and the control ($n = 31$) groups were created from the first year students of the PNU of the Faculty of Technology in order to conduct a formative pedagogical experiment. The formative experiment was conducted during 2019–2020, which introduced the issue of ecological education formation in students in the process of their physical education as well as fitness and health recreation events. The purpose of the formative pedagogical experiment was to find out the relationship between the students' ecological education and their level of health. The level of the students' physical health was assessed according to the method of H. L. Apanasenko [12].

One of the ways to improve the efficiency of the educational process of physical education, the quality of sporting and mass participation as well as fitness and health recre-

Table I. Indicators for assessing the state of ecological education of students (n = 503, points)

Specialties	Structural components of ecological education				Overall rating
	Ecological knowledge	Ecological character building	Ecological worldview	Ecological culture	
Veterinary hygiene, sanitation and expert investigation	66.4	66.1	70.3	71.6	68.6
Technology and processing of livestock products	63.6	61.2	68.4	59.6	63.2
Ecology	64.8	65.3	67.9	43.2	60.3
Veterinary medicine	51.3	59.8	56.4	67.5	58.8
Law	45.8	67.2	47.4	56.7	54.3
International economic relations	53.2	57.9	55.6	49.7	54.1
Horticulture and viticulture	47.1	56.2	53.5	53.4	52.6
Plant protection and quarantine	57.1	51.7	53.9	46.2	52.2
Forestry	45.8	52.4	53.7	56.5	52.1
Geodesy and land management	53.4	49.7	51.7	47.8	50.7
Agronomy	46.5	42.4	52.5	36.8	44.6
Public administration	42.6	45.8	44.3	41.2	43.5
Agricultural engineering	44.5	41.3	51.8	35.8	43.4
Industry engineering	39.5	40.6	43.7	38.5	40.6
Management	37.7	43.4	44.6	33.9	39.9
Economy	38.4	40.1	40.8	35.5	38.7
Information systems and technologies	38.3	40.2	41.6	31.7	38.0

ation events and maintaining the health of student youth included the introduction of the issues of ecological education in the curriculum of physical education of students, which provided students with knowledge of positive and negative impact of environmental factors on the state of human life-sustaining activities; provided recommendations for exercise and sports in a polluted environment, including various temperature factors, polluted air, water, food, etc. Three main issues were addressed: 1) to form the purpose of ecological education in physical education; 2) to determine the content and hierarchy of tasks of ecological education and character building in the field of physical culture and sports; 3) to substantiate the principles of implementation of ecological education and character building within the system of physical education of students.

Research methods: analysis and generalization of the scientific and methodological literature (29 sources on the topic of the article from the scientometric databases PubMed, Scopus, Web of Science Core Collection and others were analyzed), questionnaire survey, pedagogical observation, testing, pedagogical experiment, methods of mathematical statistics. The authenticity of the difference between the indicators of students of studied groups was determined by Student's t-test.

Ethics. The research was performed in accordance with the requirements of the Regulations on the system of academic honesty in educational and scientific activities of PNU (Protocol of the Academic Council No. 2 dated

18.02.2018) and SSPU (Protocol of the Academic Council No. 12 dated 18.06.2020).

RESULTS

It is established that the issues of ecological education and character building of students in European countries are of great importance. The most typical tasks that unite the views of most educationists in European countries on ecological education are: the formation of knowledge about the relationships patterns of natural phenomena, the unity of animate and inanimate nature, the interaction of a human being, society and nature; education of motives, needs and skills of ecologically expedient behaviour and activity, healthy way of life, desire to vigorous activity concerning protection of environment; development of intellectual and emotional spheres of students' psyche on the basis of causal analysis of ecological situations and emotional pain in relation to nature.

The multi-level system of environmental monitoring that exists in Ukraine makes it difficult to obtain and use the information needed by students to maintain health and safe life-sustaining activities. Therefore, most environmental monitoring issues are solved by departmental organizations that deal with their special tasks, which makes it very difficult for students to get a single picture of the state of pollution of the environment in which physical education sessions as well as various fitness and health recreation

Table II. Comparative analysis of the level of ecological education in the first and the fourth year students of the Faculty of Ecology of the PNU (n = 92, points)

Structural components of ecological education	First year students (n=49)	Fourth year students (n=43)	Difference
Ecological knowledge	65.9	78.5	12.6
Ecological character building	67.1	78.9	11.8
Ecological worldview	68.4	79.6	11.2
Ecological culture	47.6	74.8	27.2
General level	62.3	78.1	15.8

Table III. Dynamics of the components of ecological education and the level of health of the EG and the CG students during formative pedagogical experiment (n = 59, $\bar{X} \pm m$, points)

Investigated indicators	EG (n = 28)		Significance of the difference		CG (n = 31)		Significance of the difference	
	Before exper.	After exper.	t	p	Before exper.	After exper.	t	p
Ecological knowledge	38.6±1.17	77.4±3.74	9.87	<0.001	39.2±1.24	44.1±2.76	1.62	>0.05
Ecological character building	42.3±1.67	74.9±2.64	10.44	<0.001	41.5±1.75	47.2±2.34	1.95	>0.05
Ecological worldview	43.7±1.87	76.9±2.94	9.53	<0.001	42.2±1.67	48.3±2.73	1.91	>0.05
Ecological culture	36.4±1.14	53.8±1.83	8.07	<0.001	38.1±1.19	43.1±2.33	1.91	>0.05
Physical health level	3.14±0.72	9.77±0.67	6.74	<0.001	3.29±0.68	5.05±0.64	1.88	>0.05

events, sports and entertainment activities are conducted. There is no organization and state program of environmental monitoring in Ukraine at the present moment, which would carry out comprehensive, systematic control over all types of environmental pollution and provide up-to-date information on vital activity security. There are also many issues regarding the unification of departmental regulations on the use of environmental resources. At the same time, many specialties the students study at totally do not raise environmental issues, the study of the impact of environmental factors on human health, and so on. Our monitoring of the quality of ecological education of the third and the fourth year students of the PNU and the SSPU of different specialties showed different levels of students' mastery of the components of ecological education, which is directly related to maintaining students' health, environmental behaviour, the use of natural factors in order to improve efficiency, healthy nutrition, etc. (Table I).

The content of ecological education is based on a system of scientific knowledge that reflects the natural and scientific, social and economic, legal, moral and ethical, technical and ideological aspects of the existence of the natural environment and conditions of life-sustaining activities. In our research, ecological education of students was assessed on four indicators: ecological knowledge, ecological character building, ecological worldview and ecological culture.

Ecological knowledge as a component of environmental education is a holistic structure and consists of cognitive and active elements of learning. Cognitive elements include ecological knowledge and the formation of the inner culture of the individual, which will contribute to the har-

monization of human relations with nature. All this helps the student to realize his place in nature and to clarify his responsibility towards it. The student has the opportunity to establish a harmonious relationship with nature on this basis. The main directions of ecological education are: education in the spirit of general ideas of environmental protection and human health, acquisition of special professional knowledge about the general laws of existence of natural and anthropogenic ecosystems.

Ecological character building forms an active environmental viewpoint of the student, which is achieved through a set of environmental and ecological education, promotion of environmental behaviour. The main function of ecological character building consisted in the acquisition and accumulation by students of experience of interaction with the environment at the cognitive, sensory and emotional as well as normative levels. It is the combination of knowledge, emotional experiences and practical skills in environmental activities that allows students to make the right decisions during their stay in the natural environment.

We defined the *ecological consciousness* of the student as an organic combination of ecological knowledge, thoughts, ideas, beliefs, feelings, and aspirations. Ecological consciousness was realized in purposeful and meaningful nature protection human activities, active defence of moral values and ecological principles. Thus, ecological knowledge is the basis for the formation of ecological consciousness of the student.

The main means of forming the *ecological culture* of students was a purposeful system of ecological education, which formed the attitude towards nature, determining the allowable degree of its transformation, mastering specific

socio-natural patterns and norms of behaviour in which further human existence is possible. To develop a strategy for the formation of ecological culture, we have created and implemented new approaches and methods of ecological education (ecologization of physical education); singled out the basic qualities of the ecologically educated personality and a technique of his step-by-step formation; correlated the content of education with the real needs and interests of students; applied ecological knowledge in physical culture and health-improving activities of students.

All this helps the student to realize his place in nature and to clarify his responsibility towards it. The student has the opportunity to establish a harmonious relationship with the environment on this basis. The obtained data confirmed the opinion that the inclusion of ecological education in physical education curriculum will significantly improve the ecological education of the individual.

It was found that students majoring in veterinary hygiene, sanitation and expert investigation showed the highest level of ecological education (68.6 points), technology and processing of livestock products (63.2 points), ecology (60.3 points), veterinary medicine (58.8 points), law (54.3 points), international economic relations (54.1 points). This indicates that these specialties are directly related to ecology, hygiene, sanitation, expertise, international attitude to ecological problems of mankind, practical attitude to environmental problems and certain practical skills as future professionals. At the same time, a large number of specialists trained at Ukrainian universities do not study the problems of the impact of the environment on human health and life-sustaining activities at all.

In addition, we conducted the comparative analysis of the level of ecological education in the first (at the end of the academic year) and the fourth year students at the Faculty of Ecology, where ecological education is the basis of their future professional activities. The analysis showed a very significant difference between the comparison contingents (Table II). The fourth year students significantly improved their indicators in all comparative components of ecological education. The research showed that the study of environmental special disciplines helps to improve the knowledge needed both to improve the students' own health and in general to improve their life-sustaining activities as well as future careers.

Therefore, we believe that the inclusion of ecological education in the work programs on physical education will significantly improve the level of ecological education of students of other faculties, where special environmental disciplines are not studied, which will generally improve their life-sustaining activities and health. The conducted pedagogical formative experiment with the second year students of the Faculty of Technology of the PNU showed that the purposeful formation of ecological education has a positive effect not only on the level of ecological knowledge, but also on improving the state of health and reducing the number of students assigned to a special medical group (Table III).

The research confirmed that purposeful work on ecological education in the process of fitness and health recreation activities has a positive effect on all indicators of

students' life. Thus, the EG students significantly improved the level of all components of ecological education, as well as the level of their health ($p < 0.001$). At the same time, no significant changes were revealed in the CG ($p > 0.05$). Moreover, at the end of the experiment, the EG students showed a significantly better level in all studied indicators ($p < 0.001$), compared with the CG, which indicates the effectiveness of the introduction of ecological education to physical education of students of higher educational institutions. In addition, the number of students of the special medical group decreased from 8 to 7 persons in the EG during the pedagogical experiment, on the contrary, its number increased from 7 to 9 persons in the CG.

DISCUSSION

The organization of the educational process of student youth living in a polluted environment requires special attention to the formation of ecological education. The reason for this is: 1) lack of ecological knowledge of teachers of physical education departments; 2) inadequate material and technical base for conducting recreational and health-improving as well as preventive physical exercises; 3) inadequate medical examination, control over the health of young people and the environment; 4) reduced number of hours devoted to physical education as well as sporting and mass participation, fitness and health recreation events; 5) unsystematic control over physical development, mental and physical performance; 6) lack of scientific recommendations for physical activities in a polluted environment; 7) non-compliance with the requirements for the peculiarities of nutrition in a polluted environment; 8) lack of knowledge, skills and abilities of students on behaviour in the natural environment during sporting and mass participation as well as fitness and health recreation events, leisure and relaxation, etc. [1, 3, 13-17].

The main tasks of ecological education of students in the process of their physical education should be: preservation of a viable natural environment; radical restructuring of ecological education and character building of the individual on scientific principles; formation of a new ecological thinking and worldview in the relationship between man, society and nature; development of ecological education taking into account national heritage, formation of ecological thinking not only in narrow national, but also in planetary aspect; promotion of ecological education, which serves to improve the individual, his inner world, develops human dignity, humanism; human self-consciousness as a part of nature, connected with numerous inseparable ties, which allows to build adequate relationships with the environment [2, 3, 8, 18-21].

It is established that theoretical knowledge on ecology became the basis for activating the consciousness of students and determined the level of their needs for environmental safety. Assimilation of theoretical knowledge stimulated students to analyse facts, their generalization and conclusions, intensified their ability to retrospectively search for information, its systematization, analysis and synthesis, accumulation of individual environmental information experience. In addi-

tion, mastering the theoretical material attracts students to learn about the values of ecological education and character building, develops and forms the cultural potential of the student's personality, expands his worldview.

Ecological character building, which is provided during physical education sessions, is designed to form an active environmental viewpoint of the student, which is achieved through a set of environmental and ecological education, promotion of ecological behaviour. The main function of ecological character building is to acquire and accumulate students' experience of interaction with the environment at the cognitive, sensory-emotional and normative levels. It is the combination of knowledge, emotional experiences and practical skills in environmental activities that allows students to make the right decisions during their stay in the natural environment. The results of our research do not completely solve the problem, they only expand the results of research by other scientists [22-29].

CONCLUSIONS

1. The analysis of the quality of ecological education of the third and the fourth year students of the PNU and the SSPU of different specialties showed the insufficient level of students' mastery of the components of ecological education. Sufficient and satisfactory level of ecological education was shown only by the students whose future professional activities within the educational process is associated with the issues of ecological education (veterinary hygiene, sanitation and expert investigation, technology and processing of livestock products, ecology, veterinary medicine, international economic relations). At the same time, a large number of students of the higher educational institutions of Ukrainian do not study the problems of the environment impact on human health and life-sustaining activities at all.
2. It is established that the purposeful formation of ecological education in students during their physical education has a positive effect not only on the level of ecological knowledge, but also on improving the state of their health and reducing the number of students assigned to a special medical group. At the end of the pedagogical experiment, the level of all components of ecological education, as well as the level of health of the EG students significantly improved ($p < 0.001$). At the same time, no significant changes were revealed in the CG ($p > 0.05$). Moreover, at the end of the experiment, the EG students showed a significantly better level in all studied indicators, compared with the CG ($p < 0.001$).
3. Ecological education today is recognized as a priority in the character building of student youth, the formation of such patterns of behaviour in the natural environment that reproduce ecological development. The creation of a new paradigm of ecological education requires the reflection and disclosure of its content, forms and methods in all academic subjects. Therefore, one of the ways to improve the effectiveness of the educational process in physical education, the quality of sporting and mass participation as well as fitness and health recreation events and main-

taining the health of student youth is the inclusion of ecological education issues in physical education curricula of the higher educational institutions of Ukraine, which should provide students with knowledge of the positive and negative impact of environmental factors on the life-sustaining activities of students; provide recommendations for exercise and sports in a polluted environment.

Prospects for further research are aimed at improving the curriculum in physical education, taking into account the ecological policy of Ukraine and the world community.

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- ORCID and contibutionship:**
 Grygoriy P. Griban: 0000-0002-9049-1485^{A,E}
 Ivan M. Okhrimenko: 0000-0002-8813-5107^{B,F}
 Mykhailo S. Myroshnychenko: 0000-0002-6920-8374^{B,E}
 Oleksandr A. Tomenko: 0000-0002-1097-965X^{B,F}
 Serhii O. Matrosov: 0000-0002-9589-438X^{C,E}
 Mariia M. Rohovenko: 0000-0001-6380-3095^{A,D}
- Conflict of interest:**
The Authors declare no conflict of interest.
-
- CORRESPONDING AUTHOR**
Grygoriy P. Griban
 Zhytomyr Ivan Franko State University, Zhytomyr, Ukraine
 tel: +380973341092
 e- mail: gribang@ukr.net
- Received:** 07.06.2021
Accepted: 28.12.2021
-
- A** - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

Appendix I

Questionnaire

for assessing the state of ecological education in student youth

Dear student!

In order to study and assess the state of ecological education of student youth, we ask you to sincerely answer the questions without missing any of them. Answer the questions clearly, concisely and specifically. Our assessment of your answers will allow us to prepare guidelines to protect your health from the negative effects of environmental factors. It is your level of knowledge on ecological issues that will help us build a methodology for ecological education in the process of physical education. Your answers will be used in a generalized form for scientific purposes only. Please answer all questions as accurately as possible. We hope for your responsibility, we are sincerely grateful to you in advance.

1. General data

Please provide some information about yourself (underline as applicable):

- Last name, first name _____
- gender: male, female
- the year of your training: ▪ 1st ▪ 2nd ▪ 3rd ▪ 4th
- which educational division do you belong to: special medical, basic, sports
- age of completed years: 17 years old, 8-20, 21 and more
- Your place of residence: village, town, city

2. Assessment of ecological knowledge

1. Define ecological education.
2. Explain the content of ecological education.
3. List the tasks of ecological education.
4. What is the importance of ecological education for humans?
5. Disclose the main directions of ecological education.
6. Disclose the importance of ecological education in the field of physical culture and sports.

3. Assessment of ecological character building

1. Define ecological character building.
2. What should ecological character building shape?
3. What are the functions of ecological character building in the system of physical education of students?

4. Assessment of ecological worldview

1. Define ecological consciousness?
2. What is the basis for ecological consciousness formation?
3. List the signs of ecological consciousness.

5. Assessment of ecological culture

1. Define ecological culture.
2. Means of ecological culture formation.
3. Conditions for ecological culture formation.
4. Name the most important indicators of ecological culture formedness.

Thank you for participating in the survey!

ORIGINAL ARTICLE

THE EFFECT OF NOS3 AND AGTR1 GENOTYPES ON THE COURSE OF THE ARTERIAL HYPERTENSION FOR THE OVERWEIGHT OR OBESE PATIENTS

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Viktoriia S. Potaskalova¹, Nick V. Khaitovych¹, Larysa V. Natrus¹, Tsira B. Abdriakhimova¹, Kateryna I. Kleban¹, Vitalii Y. Lunov¹, Oleg A. Korop²

¹BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

²KHARKIV MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KHARKIV, UKRAINE

ABSTRACT

The aim: Objective of the research is to determine the effect of NOS3 and AGTR1 genotypes of patients with arterial hypertension and high body mass index in the course of the disease.

Materials and methods: 58 patients (22 men and 36 women) with AH and high BMI were examined. The average age of the examined patients was 53.6 ± 8.7 years. The analysis of rs1799983 polymorphisms of the NOS3 gene (localization 7q36.1; 7:150999023) and AGTR1 (type 1 receptor for angiotensin 2 1166 A>C) was performed using TaqMan assay (Thermo Fisher Scientific, USA) by real-time PCR (Applied Biosystems, USA) using TaqMan probe amplification products. Genomic DNA samples were isolated from stabilized blood using a Genomic DNA Mini Kit reagent (Invitrogen, USA). The Statistica 10 program (StatSoft Inc.) was used for statistical processing of the obtained data, USA). The independent samples were compared using the Mann-Whitney (U) criterion. In all cases of statistical evaluation, the reliability of differences was taken into account at a value of $p < 0.05$.

Results and conclusions: Polymorphism of the NOS3 and AGTR1 genes is associated with early development and complicated course of cardiovascular pathology. The combination of NOS3 and AGTR1 gene polymorphism in patients with the high body mass index increases the risk of complications in hypertension. Using a mathematical model to predict the probability (95%) of genetic mutations in two genes (NOS3 and AGTR1) increases the effectiveness of diagnosis for patients with the high risk of developing cardiovascular complications.

KEY WORDS: arterial hypertension, NOS3 and AGTR1 genotypes, body mass index

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INTRODUCTION

The cardiovascular diseases, as arterial hypertension (AH), determine the mortality and disability of the adult population. The number of the patients with AH increases with age, which is associated with a violation of the response to vasodilating factors, this problem leads to the development of vascular diseases, accompanied by pathological vascular remodeling, impaired tissue perfusion. An early predictor of atherosclerosis and complications of cardiovascular pathology is endothelial dysfunction. Vascular endothelium supports hemostasis, and endothelial dysfunction affects vascular tone, immune inflammation, and platelet activation [1, 2]. During the aging process of the endothelium, both structural and functional changes occur, in particular, angiogenesis, vascular wall tone are disrupted, and mitochondrial dysfunction occurs. In addition, endothelial disorders are affected by oxidative stress, hyperuricemia, activation of the renin-angiotensin system, and vascular inflammation [3-6].

The disorders of NO synthesis by endotheliocytes may be genetically determined. The replacement of guanine with thymine at 894 positions in exon 7 of the NOS3 gene leads

to a change of the enzyme activity due to a change in the amino acid sequence (glutamine is replaced with arginine at 298 positions) [7-10]. The existing T allele in patients with AH and a high body mass index (BMI) is associated with an early (on average 4.5 years) development of the disease, which was found among 39.7% of patients.

The gene AGTR1 polymorphism is associated with high vascular stiffness [11] and endothelial dysfunction [12] and cardiovascular complications in pathology, which also affects the development and severity of hypertension [13-15].

Therefore, better understanding of the pathophysiology of cardiovascular diseases can be useful for optimizing prevention and treatment, and patients with AH will be provided optimal blood pressure (BP) control. Routine examinations of patients with AH can identify damage of the target-organs and determine the risks of complications, analyze the effectiveness of antihypertensive therapy, but the examinations do not show additional information on the personal treatment of such patients. A data variety about the contribution of the NOS3 and AGTR1 genes polymorphisms to the development of the disease and its complications, and the course of concomitant pathology,

in particular the 2nd type of the diabetes, obesity, hypercholesterolemia, chronic renal failure, are aimed to find a pharmacogenetic basis for the treatment choice strategy for patients with AH [16-24].

THE AIM

Objective of the research is to determine the effect of NOS3 and AGTR1 genotypes of patients with arterial hypertension and high body mass index in the course of the disease.

MATERIALS AND METHODS

58 patients (22 men and 36 women) with AH and high BMI were examined. The average age of the examined patients was 53.6 ± 8.7 years. The analysis of rs1799983 polymorphisms of the NOS3 gene (localization 7q36.1; 7:150999023) and AGTR1 (type 1 receptor for angiotensin 2 1166 A>C) was performed using TaqMan assay (Thermo Fisher Scientific, USA) by real-time PCR (Applied Biosystems, USA) using TaqMan probe amplification products. Genomic DNA samples were isolated from stabilized blood using a Genomic DNA Mini Kit reagent (Invitrogen, USA). The Statistica 10 program (StatSoft Inc.) was used for statistical processing of the obtained data, USA). The independent samples were compared using the Mann-Whitney (U) criterion. In all cases of statistical evaluation, the reliability of differences was taken into account at a value of $p < 0.05$.

RESULTS AND DISCUSSION

The Endothelial nitric oxide synthase (NOS3) is a dimeric enzyme, the activity and expression of which are regulated at the transcriptional, post – transcriptional, and post-translational levels. The gene encoding NOS3 includes polymorphic sites (single-nucleotide polymorphisms, tandem repeats, microsatellites, and inserts). Nitric oxide synthesis may be affected by some polymorphisms due to NOS3 activity or expression. NOS3 haplotypes can increase the risk of developing diseases [25] based on endothelial dysfunction, in particular ischemic heart disease (CHD), myocardial infarction, ischemic brain stroke, hypertension, and chronic kidney disease [26-31] (table. I).

The data of the research of gene polymorphism and the link between them with risks for patients with a Cardio-logical profile alter in different populations. Even on the territory of the same continent and the same country, depending on the region, the population may have different genotypes of the same gene, so it is not possible to extrapolate statistics on the polymorphism of a particular gene to the entire population of the country.

Mutations in the Nos3 gene can become predictors of the 2nd type diabetes mellitus (DM) [32, 33], although the dominant AG genotype of the NOS3 rs1800779 and T2D polymorphisms is protective [34], while the GT polymorphism of the NOS3 gene is associated with the development of arterial hypertension in Brazilian women [35]. The presented meta-analysis data in the European

population according to Mendelian randomization indicate an association of exon polymorphism in NOS3 (rs1799983, p.Glu298Asp) and in intron COL4A1 (rs9521634) and near DYRK1A (rs720470) with ischemic brain stroke due to changes in blood pressure levels [36]. In patients in the Antalya population, the NOS3 GT and TC polymorphism (rs2070744) is associated with hypertension [37].

Also, a prospective study shows the role of nos3 gene polymorphism in the formation of cerebral artery aneurysms, their rupture or the development of vasospasm, and the existing polymorphism can be considered a risk factor for the development of vascular complications along with smoking, hypertension, and diabetes [38]. The RS2070744 NOS3 polymorphism may be considered a factor of genetic predisposition in Sudan to hypertension [39].

A high risk of CHD was shown in Caucasians, South Asian populations, and Middle Eastern people with the existing TT and GT nos3 polymorphism. TT polymorphism significantly increases the risk (odds ratio >2) of developing ASF in 10 countries (Ukraine, Brazil, Great Britain, Egypt, India, Iran, Chile, South Korea, Morocco, Japan, etc.) [40]. A strong correlation exists in the TG Enos and AG polymorphism in Morocco [41]. Conflicting data on NOS3 polymorphism and the risk of myocardial infarction. A meta-analysis revealed an association of TC polymorphism in the NOS3 gene with the risk of myocardial infarction in Asian and European populations [42]. In patients with CHD from Pakistan, the Glu298Asp variant of the NOS3 gene showed no association with hypertension and dyslipidemia, but had a strong correlation with systolic blood pressure [43]. NOS3 polymorphism in Iranian patients is associated with the development of multiple sclerosis [44], GT, TC, and 4a/4b polymorphisms were not associated with the risk of CHD in residents of northern Iran [45]. Patients with CHD are more likely to have the rs1799983 t and rs2070744 G alleles than the GG genotype, and diastolic blood pressure increases with increasing BMI [46].

Among the examined patients, the wild allele of the NOS3 gene was found inside 35 (60.3%) individuals, the GT genotype - inside 20 (34.5%) patients, and the TT genotype – inside 3 (5.2%) subjects. It is known that the TT genotype increases the risk of hypertension by 2.3 times: the no level is lower in patients with hypertension and diabetes with GT and TT polymorphism [47, 48].

The early development of CHD with a gt gene polymorphism was found [49-51], although among the Tunisian population, the GT polymorphism is not associated with the development of CHD [52].

The pathogenesis contribution arguable data of cardiovascular diseases are presented in numerous literature reviews of the polymorphism of the AGTR1 gene. Thus, GT (rs275652) and AG (rs275653) polymorphisms are associated with severe atherosclerotic vascular damage among patients in the Mexican population [53, 54] (table. II).

Also, polymorphism of the AGTR1 gene causes sodium reabsorption in the distal tubules and the development of vascular stiffness, regardless of the level of blood pressure [55, 56]. The activation of renin-angiotensin of the aldoste-

Table I. Association of NOS3 gene polymorphisms with the development of pathological conditions

Genotype	Result	Reference
NOS3 gene polymorphism	Cerebral artery aneurysms, rupture or development of vasospasm; disseminated sclerosis	Subhas K. Konar etc., 2019; Mohammad Mehdi Heidari etc., 2017;
TT polymorphism of the NOS3 gene	AH	Jelita Siregar etc., 2020;
GT polymorphism of the NOS3 gene	AH; the early development of IXC	Abel Barbosa Lira Neto etc. 2019; Sanaa Nassereddine etc., 2018; Boqian Zhu etc., 2017; Khalil Mahmoodi etc. 2016; Sherif Arafa etc., 2018;
Alleles rs179983 T and rs2070744 G of the NOS3 gene	Increased diastolic blood pressure among patients with high BMI	G L Zhao and etc, 2016;
NOS3 haplotypes G894T/T-786C	Reduced NO level among patients with hypertension and diabetes; CHD; myocardial infarction; ischemic cerebral stroke; hypertension; chronic kidney disease	Robin Johns etc, 2018; Omneya Moguib etc, 2017; Jelita Siregar etc, 2020; Cecilia Vecoli, 2014; Alejandro Marín Medina etc, 2018; E A Trifonova etc, 2019; N Yu etc, 2019; Beáta Soltész etc; Gustavo H Oliveira-Paula etc, 2016; Xiang-Zhen Kong etc, 2017; Dalia El-Lebedy etc, 2018; Süleyman Ömer Anlıaçık etc, 2019; Sahar Gamil etc, 2017; Rainer Malik etc, 2018;
AG polymorphism NOS3 rs1800779 and T2D	High systolic blood pressure	Saleem Ullah Shahid etc, 2017;

Table II. Association of agtr1 gene polymorphisms with the development of pathological conditions

Genotype	Result	Reference
AGTR1 gene polymorphism	Increased vascular stiffness; Hypoxia resistance	Marcin Cwynar etc, 2016; Tatiana I Baranova etc, 2017;
Agtr1 GT (rs275652) and agtr1 AG (rs275653) polymorphisms	Systemic atherosclerosis	Tatiana S Rodríguez-Reyna etc, 2016; Zhongping Shi et al., 2021;
genotype CC AGTR1	High renin levels; Complications of concomitant diseases	Tatyana Mulerova etc, 2020; Ana Céla Sousa etc, 2018; Keping Chen etc, 2021; Samantha Kohli etc, 2016; Sandrita Simonyte etc, 2017; Benjamin Goldstein etc, 2016; Sudhir Jain etc, 2018; Elena V Zholdybayeva etc, 2016; Roseline Wai Kuan Yap etc, 2017; Hsien-Feng Chang etc, 2018;
AC polymorphism of the AGTR1 gene	Liver damage; Insulin resistance; Endothelial dysfunction; Adipokine activation; Diabetic nephropathy; Hypertriglyceridemia; Increased low-density lipoproteins	Giovanni Musso etc, 2019; Dana de Gracia Hahn etc, 2019; Yan Zhuang etc, 2018; H-L Xu etc, 2020; Xun Li etc, 2016;
Allel C in rs5186 of the AGTR1 gene	High mortality and cardiovascular complications among patients with end-stage kidney disease	Sharon M Moe etc, 2019.

rene system (RAAS) is connected with AH. The results of a meta-analysis of the genetic association between RAAS genes and chronic kidney disease indicated a reduced risk of kidney damage in the presence of the AGT rs699-T allele and the AGTR1 rs5186-a allele [57], which also depends on the population [58]. The genetic polymorphism of AGTR1

in many researches is not associated with the development of hypertension [59, 60], although the CC genotype of this gene is associated with high level of renin, which contributes to the development of hypertension, at least in some populations [61-65] and negatively affects the course of concomitant diseases [66-69].

The collected data result for the dominant type of AA homozygote of the AGTR1 gene was detected inside 39 (67.2%) patients, and the heterozygous AC polymorphism was detected inside 19 (37.8%) of the examined patients.

A number of researches have shown no association between AGTR1 gene polymorphism and insulin resistance [70], although another study found the effect of AGTR1 AC polymorphism on liver damage, the development of insulin resistance and endothelial dysfunction, the effect on the activation of adipokines, chemokines and pro-inflammatory cells in response to fat consumption [71, 72], which is important while choosing drugs. The AGTR1 gene mutation is associated with the development of diabetic nephropathy among the Asian population [73]. The research result linked the existing polymorphism to high levels of triglycerides and low-density lipoproteins [74, 75]. The C allele in rs5186 of the AGTR1 gene is associated with high mortality and cardiovascular complications in patients with end-stage kidney disease, both in the European and African populations [76]. Also, according to other authors, polymorphism of the AGTR1 gene can cause resistance to hypoxia [77].

It is known about the link of myocardial remodeling with AH, which is determined among patients with hypertension to determine the stage and control the course of the disease, namely, the relative thickness of the left ventricle (VTS LV), left ventricular myocardial mass (MMLSH), and the left ventricular myocardial mass index (MMLSH) [78, 79]. Hypercholesterolemia is also a risk factor for hypertension and requires pharmacological correction in overweight patients, even at normal blood pressure values [80-82]. Hyperuricemia increases the risk of developing complications of hypertension, and it is advisable to determine the level of uric acid (uric acid) in all patients with increased blood pressure [83-85]. Both hypertension and obesity are associated with increased immune inflammation, which promotes remodeling of the vascular wall and increases its stiffness [86-88]. Obese patients have a high risk of developing insulin resistance and diabetes of the 2nd type [89-91].

According to the results of the analysis of indicators obtained during the examination of patients with AH, the heart rate (HR) has a likely relationship with the prediction of the existing polymorphism of the NOS3 and AGTR1 genes.

As a result of performing logistic regression to model the differentiation of existing polymorphism by NOS3 or AGTR1 genes separately, no statistically significant results were found. However, with the simultaneous polymorphism of these genes, we obtained strong correlations with the given risk factors for the course of hypertension. we created a mathematical equation according to which the probability of genetic mutations for two genes (NOS3 and AGTR1) can be predicted with a probability of 95%:

$$Y = 38,8 \times \text{BLP HD} + 21,8 \times \text{BLP LD} + 0,75 \times \text{BLP LD} + 5,6 \times \text{AI} - 0,009 \times \text{ILVMM} - 0,069 \times \text{LVMM} + 0,86 \times \text{N} + 0,009 \times \text{urinary capacity} + 0,21 \times \text{HD AH} - 1,84 \times \text{TC} + 2,8 \times \text{FBG} + 0,006 \times \text{HR} - 122,1$$

Where:

Y is the theoretical probability of mutations;

BLP HD - high-density beta-lipoproteins;

BLP LD - low-density beta-lipoproteins;

AI - atherogenicity index;

N – neutrophil count;

HD AH - hypertension-duration of arterial hypertension;

TC - total cholesterol;

FBG - fasting blood glucose;

HR - heart rate;

LVMM - left ventricular myocardial mass;

ILVMM - left ventricular myocardial mass index;

The inclusion in the formula of the indicator – the duration of the disease, can become a criterion for an early prognosis of the AH development in this category of patients.

The definition of gene polymorphism is associated not only with the risk of developing the disease, in particular, certain gene mutations can be protective in nature. Information about the gene polymorphism helps in choosing pharmacogenetic treatment for patients with the high risk of developing cardiovascular complications.

Since these indicators can be determined while examining a patient at the primary level and do not require high economic costs, this formula will help identify individuals with the likely presence of polymorphism for two genes (NOS3 and AGTR1) and, if need, send them for additional genetic examination to correct the treatment.

CONCLUSIONS

1. Polymorphism of the NOS3 and AGTR1 genes is associated with early development and complicated course of cardiovascular pathology.
2. The combination of NOS3 and AGTR1 gene polymorphism in patients with the high body mass index increases the risk of complications in hypertension.
3. Using a mathematical model to predict the probability (95%) of genetic mutations in two genes (NOS3 and AGTR1) increases the effectiveness of diagnosis for patients with the high risk of developing cardiovascular complications.

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ORCID and contributionship:

Viktoriia S. Potaskalova: 0000-0002-6255-7513 ^{A,E}
 Nick V. Khaitovych: 0000-0001-6412-3243 ^{B,F}
 Larysa V. Natrus: 0000-0003-1763-0618 ^{B,C}
 Tsira B. Abdriakhimova: 0000-0002-9723-3067 ^{C,F}
 Kateryna I. Kleban: ^{B,F}
 Vitalii Y. Lunov: 0000-0002-7085-8454 ^{B,E}
 Oleg A. Korop: 0000-0002-8833-4296 ^{B,E}

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CORRESPONDING AUTHOR

Viktoriia S. Potaskalova

Bogomolets National Medical University

13 Taras Shevchenko Boulevard, 01601 Kyiv, Ukraine

e-mail: doktorviktorya@gmail.com

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REVIEW ARTICLE

ON THE NEED TO IMPROVE THE SYSTEM OF PREVENTION OF ALCOHOL AND DRUG ADDICTION AMONG SERVICEMEN OF THE DEFENSE FORCES

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Boris I. Palamar¹, Lyudmila A. Ustinova², Natalia V. Kurdil², Irina V. Ogorodniychuk²¹BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE²UKRAINIAN MILITARY MEDICAL ACADEMY, KYIV, UKRAINE**ABSTRACT**

The aim: Analysis of the legal framework for the development of scientifically sound measures for the prevention and detection of drug addiction and substance abuse in the Armed Forces of Ukraine as an important element of the system of medical support for combat readiness of troops.

Materials and methods: The normative documents on carrying out obligatory preventive narcological examinations in the Armed Forces of Ukraine and other power ministries and departments are considered.

Conclusions: Today in Ukraine it is necessary to improve the legal framework and strengthen control over the work of military commissariats to study conscripts, collect the necessary information about alcohol and drug addiction of citizens who are called up to the Armed Forces of Ukraine.

KEY WORDS: alcohol dependence, drug addiction, preventive medicine, military medicine

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INTRODUCTION

Today, in some parts of Donetsk and Luhansk regions, as well as in the Autonomous Republic of Crimea and the city of Sevastopol, the state authorities of Ukraine do not exercise their powers, which significantly affect the drug situation not only in these regions but in the country as a whole. Events in the area of the Anti-Terrorist Operation (from April 14, 2014 to April 30, 2018) in the Donetsk and Luhansk regions necessitated the announcement of mobilization to increase the number of the Armed Forces of Ukraine (hereinafter – the Armed Forces). In the first six waves of mobilization of the Armed Forces alone, 210,000 servicemen were recruited, and some of them signed a contract and today continue their military service in the Joint Forces operation (from April 30, 2018 to the present), mostly in the newly formed military parts.

The modern army is a part of society, and all processes, both positive and negative, are reflected in military service. The prevalence of alcoholism and drug addiction among the civilian population determines the presence of these phenomena among the military.

Alcohol and drug addiction as dangerous phenomena penetrate the military environment, especially with young people who have become addicted in school and adolescence. The consequence of alcohol and drug addiction is deviant behaviour, which in combat significantly increases the risk of non-combat losses.

In total, since the beginning of the Anti-Terrorist Operation, the number of servicemen whose deaths were not

directly related to the fighting has exceeded 1,300. Some non-combat casualties are related to alcohol and drug abuse among military personnel. For example, in the first half of 2017, drug-related crimes accounted for 5.6% (78) of the total. In the anti-terrorist operation zone, during the same period, they accounted for 15% (42) of the total number of crimes.

The above became the basis for the generalization of the modern regulatory framework on the organization of prevention, in particular the detection of alcoholism and drug addiction among servicemen of the Defense Forces.

THE AIM

Analysis of the legal framework for the development of scientifically sound measures for the prevention and detection of drug addiction and substance abuse in the Armed Forces of Ukraine as an important element of the system of medical support for combat readiness of troops.

MATERIALS AND METHODS

An analysis of publications related to problematic issues in the field of detection and prevention of alcohol and drug addiction among servicemen. The normative documents regulating the procedure for conducting obligatory preventive narcological examinations and examinations in the Armed Forces of Ukraine and other power ministries and departments are considered.

REVIEW AND DISCUSSION

Monitoring of the drug and alcohol situation in Ukraine is carried out in accordance with the Resolution of the Cabinet of Ministers of Ukraine of July 10, 2019 № 689 “Issues of monitoring the drug and alcohol situation in Ukraine” [1] and the Order of the Cabinet of Ministers of Ukraine of 06.02.2019 № 56-r “On approval of the action plan for 2019-2020 for the implementation of the State Drug Policy Strategy for the period up to 2020” [2].

Public information on the results of the annual monitoring is available on the website of the state institution “Center for Mental Health and Monitoring of Drugs and Alcohol of the Ministry of Health of Ukraine”. According to the latest state monitoring of drugs and alcohol of the Ministry of Health of Ukraine, at the beginning of 2019 in Ukraine were registered more than 9 thousand people who were diagnosed for the first time in their lives with mental disorders related to drug use, among their share of persons aged 15 to 35 was 75.1%. The main users of drugs are men (85.2%); the share of women - 14.8%; residents of cities - 83.7%, rural areas - 16.3%. The highest prevalence of drug addiction (per 100 thousand population) was recorded in Zaporizhzhia (364.61), Kropyvnytskyi (262.2), Odessa (235.47), Mykolaiv (213.93), Dnipropetrovsk (210.3), Kherson (181.7), Chernihiv (165.93) regions and the city of Kyiv (293.87). Among the most consumed drugs, opioids account for 65.5%; cannabinoids - 9.7%; cocaine - 0.02%; hallucinogens - 0.08%; simultaneous use of several drugs and other psychoactive substances - 24.2% [3].

Thus, according to official data, at the beginning of 2019, more than 100,000 drug addicts were registered across the country. However, according to experts of the international project “European survey of students on alcohol and other drug use - ESPAD”, given the hidden nature of this phenomenon, drug latency, researchers believe that the idea of the number of drug addicts in Ukraine is 450-500 thousand people is more than 1% of the total population. According to opinion polls conducted within the framework of this project, 35% of freshmen in colleges and 25% of university students have experience of drug use. Experts believe that the number of Ukrainians who cannot live without drugs is increasing by almost 8% every year [4].

In 2017, the Verkhovna Rada of Ukraine adopted the Law “On Amendments to Certain Legislative Acts of Ukraine Concerning Strengthening the Responsibility of Servicemen and Certain Other Persons” [5]. The law stipulates that the consumption of alcoholic, low-alcohol beverages or the use of narcotic drugs, psychotropic substances or their analogues by servicemen, conscripts and reservists during gatherings on the territory of military units, military facilities, or the appearance of such persons on the territory of a military unit while intoxicated state, in a state of narcotic or other intoxication, or their performance of military duties in a state of intoxication, in a state of narcotic or other intoxication, as well as the refusal of such persons to undergo an examination for alcohol, drug or other intoxication entail the imposition of a fine of seventy to one hundred and forty-five non-taxable minimum incomes or arrest with detention on guard duty for up to five days. It is provided that the participation of chiefs (commanders) and other leaders in such actions entails the imposition of a fine of one hundred forty-five to two hundred and fifteen tax-free minimum incomes or arrest with detention on guard duty for a period of five to seven days. Such acts committed during a special period

or by a person who during the year was subject to an administrative penalty for the same violations, entail the imposition of a fine of two hundred and fifteen to two hundred and eighty-five non-taxable minimum incomes or arrest with detention on guard duty for a period of seven to ten days.

Analysis of crimes related to drugs and psychotropic substances among servicemen shows that in the structure of seized drugs and psychotropic substances were most often found: products of cannabis processing in the form of crushed parts of this plant (hashish, anasha, marijuana) or related to bouquet; hemp resins (black or brown tablets); products of opium poppy processing in the form of raw opium; heroin; synthetic drugs (methadone, amphetamine, opium alkaloids); pharmaceuticals; precursors (acetic anhydride, ephedrine, lysergic acid, etc.) [6, 7].

In Ukraine, in order to timely identify patients with alcoholism and drug addiction, and establish medical contraindications to certain activities, a preventive drug test has been introduced.

The procedure for drug examination is determined by:

- Resolution of the Cabinet of Ministers of Ukraine № 1238 of 06.11.1997 “On mandatory preventive narcological examination and the procedure for its conduct” (hereinafter - Resolution № 1238) [8];

- Order of the Ministry of Health of Ukraine № 339 of 28.11.1997 “On improving the system of preventive anti-alcohol and anti-drug measures and mandatory preventive drug examinations” (hereinafter - Order № 339) [9];

- Order of the Ministry of Internal Affairs of Ukraine № 1296 of 04.11.2003 “On medical care in health care facilities of the Ministry of Internal Affairs of Ukraine” [10];

- Order of the Ministry of Internal Affairs of Ukraine № 1340 of 11.12.2014 on amendments to the order of the Ministry of Internal Affairs of Ukraine of 04 November 2003 № 1296 [11];

- Order of the Security Service of Ukraine № 310 of 17.07.2012 “On the procedure for drug and psychiatric examinations in the Security Service of Ukraine” [12].

Regulatory documents provide for the passage of primary, periodic and extraordinary drug examinations. The initial examination is carried out by persons who are employed in a certain position. Periodic review is conducted at regular intervals (different ones for different professions) throughout the period of work in his position. The issue of extraordinary examination is decided individually.

A narcologist who has a certificate in the specialty “Narcology” has the right to conduct a narcological examination. Specialists who have undergone special training according to the program approved by the Ministry of Health of Ukraine, testing and received a Certificate in the form № 146/0 are allowed to work. Training on modern drug testing should take place every three years. Unauthorized narcologist cannot perform preventive examinations.

In case of detection of signs of narcological disease or in case of disagreement with the results of preventive narcological examination, a citizen has the right to undergo additional narcological examination, psychological testing and medical laboratory examination in an inpatient department of a narcological hospital within ten days.

The procedure for preventive narcological examination was prescribed in the order of the Ministry of Health of Ukraine “On approval of clinical protocols for medical care for “Addic-

tion" from 21.09.2009 № 681. According to this order, the final conclusion on the suitability or unsuitability of the person to perform certain work is carried out by the medical-consultative commission consisting of 3 narcologists; the examination procedure lasts up to 10 days [13]. However, this order expired, according to the order of the Ministry of Health of Ukraine dated 28.02.2020 № 590 [14].

After the preventive narcological examination and additional examination in the hospital, the citizen is issued a certificate of the results of the narcological examination (form № 140-0 (registration). Certificate a single document (according to the Order of the Ministry of Health of Ukraine № 339 of 28.11.1997), certifying the presence or absence of contraindications to the performance of functional duties and activities for which the passage of primary and periodic preventive drug testing is mandatory [9].

According to paragraph 17 of the "List of professions and activities for which primary and periodic preventive drug testing is mandatory" approved by the Cabinet of Ministers of Ukraine № 1238 of 06.11.1997 [8], in order to timely identify patients with alcoholism, drug addiction, drug addicts, Persons entering the Ministry of Defense of Ukraine, including those called up for military service, are subject to preventive narcological examination. The Ministry of Health of Ukraine has identified narcological establishments as the venue for the preventive narcological examination. The list of state health care facilities where a narcological examination can be performed is approved by the Ministry of Health of Ukraine. The list of public health facilities where a drug test can be performed is approved by local government administrations [10].

A significant part of Ukrainian citizens undergo this procedure in a narcological institution at their place of permanent residence, except for employees of: the Ministry of Internal Affairs; Security Services of Ukraine; Foreign Intelligence Service of Ukraine; State Border Guard Service of Ukraine. Persons working in the above structures undergo drug examinations in departmental medical institutions.

Thus, narcological examinations in the Security Service of Ukraine are conducted by narcologists of medical and preventive institutions of the Security Service of Ukraine, narcological examinations during medical examinations are conducted by narcologists who are members of the military medical commission.

Mandatory periodic and extraordinary narcological examinations of privates and officers of the Ministry of Internal Affairs of Ukraine, employees of bodies and subdivisions of internal affairs, regardless of subordination, are conducted by psychiatrists of medical centers of the Ministry of Internal Affairs of Ukraine.

It should be noted that the organization of mandatory preventive narcological examination of citizens and persons entering the Ministry of Defense of Ukraine and serving in the military in departmental treatment and prevention facilities is not regulated.

In addition, if it is necessary to conduct extraordinary examinations of privates and officers, law enforcement officers, servicemen, employees of the National Guard of Ukraine and in case of impossibility of conducting such examinations in departmental medical institutions, these persons are sent for extraordinary examinations to state and municipal security health institutions, where an extraordinary examination of a person is carried out on a paid basis at the expense of the customer (department). If

a person has passed a narcological examination in a medical institution that is not included in the approved list, the certificate of the results of such examination is not valid.

According to the Resolution of the Cabinet of Ministers of Ukraine № 1238 of 06.11.1997 [8] preventive narcological examination is carried out according to the mandatory program, which includes drug narcological examination, psychological testing and medical laboratory examination in the manner prescribed by the Ministry of Health of Ukraine.

According to the Order of the Ministry of Health of Ukraine № 507 of December 28, 2002 "On approval of standards for medical care and quality indicators of medical care" [15], laboratory (toxicological) analysis should consist of 2 stages: preliminary diagnosis (toxicological screening) and final diagnosis (chemical-analytical methods: thin-layer, gas-liquid, high-performance liquid chromatography with mass spectrometry).

Performing two stages of toxicological research increases the reliability of the final conclusion on the presence of a narcotic or psychotropic substance and meets international standards. Today, the procedure for the use of rapid tests for alcohol and drugs during drug examinations remains unregulated. Positive results of such tests, in accordance with the requirements of the Ministry of Health of Ukraine, are not considered grounds for termination of a person's performance of official duties.

The Armed Forces of Ukraine do not have military medical institutions that have the right to conduct preventive drug examinations in full, namely, there are no laboratories that have the right to conduct medical laboratory examinations in the manner prescribed by the Ministry of Health of Ukraine. Therefore, to ensure compliance with current legislation on the procedure of drug testing, the medical service of the Armed Forces of Ukraine in case of need for special drug tests of servicemen during military service, organizes drug tests on a self-supporting basis in state and municipal health care facilities. The list of facilities where you can undergo a drug test is approved by order of the Ministry of Health of Ukraine, and at the regional level - by local state administrations.

CONCLUSIONS

Based on the study of the legal framework of Ukraine in the field of prevention of alcohol and drug addiction, in particular the procedure for organizing and conducting preventive drug examinations, it is established that certain issues of organization of prevention and detection of alcohol and drug addiction among servicemen of the Defense Forces are finally unresolved.

Based on the data obtained, we consider it necessary: development and approval of an interdepartmental order on the procedure for conducting narcological and psychiatric examinations in the Armed Forces of Ukraine; development and approval of an interdepartmental order on coordination of actions of specialists of the Ministry of Health of Ukraine and the Ministry of Defense of Ukraine in providing psychiatric and medical-psychological assistance to participants of the Anti-terrorist operation/Joint Forces Operation; strengthening control over the work of military commissariats on the study of conscription contingents, gathering the necessary information on alcohol and drug addiction of citizens who are called up to the Defense Forces.

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ORCID and contributionship:

Lyudmila A. Ustinova: 0000-0002-6582-7231 ^{A,B}Natalia V. Kurdil: 0000-0001-7726-503X ^{A, B,D}Boris I. Palamar: 0000-0003-2510-0713 ^{A, B,D- F}Irina V. Ogorodnychuk: 0000-0003-1063-1829 ^E

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Borys I. Palamar

Bogomolets National Medical University

13 Taras Shevchenko Blvd., 01601 Kyiv, Ukraine

tel: +380672387654

e-mail: palamar.bi@ukr.net

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A - Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis,
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REVIEW ARTICLE

THE USE OF ULTRASOUND GUIDED HIGH INTENSITY FOCUSED ULTRASOUND (HIFU) IN THE TREATMENT OF UTERINE FIBROIDS: AN OVERVIEW

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Yoana Ivanova¹, Dobromir Dimitrov², Kameliya Dimitrova³, Aparajeya Shanker³, Angel Yordanov¹¹DEPARTMENT OF GYNECOLOGIC ONCOLOGY, MEDICAL UNIVERSITY PLEVEN, PLEVEN, BULGARIA²SURGICAL ONCOLOGY DEPARTMENT, MEDICAL UNIVERSITY PLEVEN, PLEVEN, BULGARIA³FACULTY OF MEDICINE, MEDICAL UNIVERSITY PLEVEN, PLEVEN, BULGARIA

ABSTRACT

Uterine fibroids are the most common benign gynecological neoplasms, with a higher prevalence in women aged between 30 and 50 years old. Fibroids may be asymptomatic, but in some cases, they can affect seriously the quality of life of the patients. In some cases, we can recommend expectant management for asymptomatic patients. Management depends on the size and location of fibroids, the age of the patient, symptoms, desire for future childbearing and the experience of the gynecologist. Medical therapy includes hormonal contraceptives, tranexamic acid, and nonsteroidal anti-inflammatory drugs (reduce heavy menstrual bleeding). Gonadotropin-releasing hormone agonists or selective progesterone receptor modulators are used mostly preoperatively. Surgical treatment includes hysterectomy, myomectomy – invasive and minimally invasive. Non-surgical management include uterine artery embolization, and focused ultrasound surgery. This review aims to present the role of High-Intensity Focused Ultrasound in the treatment of uterine fibroids.

KEY WORDS: uterine fibroids, Focused Ultrasound Surgery, High-Intensity Focused Ultrasound, Ultrasound guided High-Intensity Focused Ultrasound

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INTRODUCTION

Uterine fibroids (UF) are the most common benign tumour pathology in women. The incidence of UF is associated with age, with a higher incidence in women between 30 and 50 years [1]. UF affects between 70-80% of all women, as they could be symptomatic or in the most cases – asymptomatic. When symptomatic, patients with UF present with a range of symptoms such as abnormal uterine bleeding, anemia, feeling of pelvic pressure and / or pelvic pain, infertility dyspareunia, constipation, pollakiuria, frequent nocturnal urination, symptoms of overactive urinary bladder [2]. In some patients, the UF may present with ureteral compression with resulting hydronephrosis and pyelonephritis [3].

The pathophysiology of UF has not been discovered. Existing theories indicate the combined effect of estrogen, progesterone and insulin-like growth factors may interact together to create conditions in the uterus for fibroid formation [4]. There is a hypothesis that the growth of UF depends on the difference between content of oestrogen receptors in endometrium and myometrium. If the concentration is lower in the myometrium than the endometrium, this oestrogen may contribute to tumour enlargement by increasing the production of extracellular matrix. There is a data that progesterone also affects UF, especially in young women, because increases the mitotic activity of myomas.

Tumour enlargement may be provoked by down-regulating apoptosis in the fibroids [5].

Modern treatment approaches include the usage of medical management, surgical management – which could be invasive and minimally invasive and non-surgical methods. Surgical methods include hysterectomy (vaginal, abdominal, laparoscopic or robotic), myomectomy (hysteroscopic, laparoscopic, robotic or by laparotomy), while non-surgical management is presented by procedures like uterine artery embolization and focused ultrasound surgery [5].

The choice of treatment depends mostly on patient preference with special regard to childbearing, choice of preserving uterus, chance of success of achieving treatment milestones, improving symptoms and overall health status of the patient [6]. The medical management of uterine fibroids include anti-fibrinolytic agents, non-steroidal anti-inflammatory drugs (NSAIDs), combined hormonal contraceptives, progesterone-only treatments, selective progesterone receptor modulators (SPRMs), anti-progestins, aromatase inhibitors, and gonadotropin releasing hormone (GnRH) agonists or antagonists [6]. Although other pharmacologic classes are being studied, current data does not show evidence of symptom or clinical improvement [6]. The fibroids characteristics, such as number, size and location, symptoms, patients age and fertility desire, determine the type of treatment, especially

when considering minimally-invasive versus open surgery and myomectomy versus hysterectomy [7, 8]. Non invasive methods like Focused ultrasound surgery (FUS), are divided in two main groups MRI (Magnetic Resonance Imaging) guided and Ultrasound guided High Intensity Focused Ultrasound (USgHIFU) [9].

THE AIM

This review aims to present the role of High-Intensity Focused Ultrasound in the treatment of uterine fibroids, the importance of searching for new non-invasive ways for treatment, to reveal the mechanisms and the safety protocols, and also to compare it with the surgical treatment of uterine fibroids.

REVIEW AND DISCUSSION

HISTORICAL PERSPECTIVES AND FUTURE LANDSCAPES OF FOCUSED ULTRASOUND

The use of focussed ultrasound begins with the discovery of ultrasound as a therapeutic modality in 1930, when the thermal effects of ultrasound on tissue were discovered [10]. In particular, ultrasound was used in physiotherapy, where the newly discovered thermal effects of ultrasound were used to treat tendonitis, synovitis and bursitis [10]. The use of focused ultrasound was pioneered by the experimentation of the Fry brothers, who, in the 1950s, experimented with the use of focussed ultrasound waves for the treatment of Parkinson's disease [11].

The subsequent years of experimentation led to the development of newer devices and newer areas of possible application of High-Intensity Focused Ultrasound (HIFU). Currently, HIFU is used in gynaecology for the treatment of UF and also in the treatment of benign, non-neoplastic diseases of the vulva [12, 13]. The use of HIFU in prostate hyperplasia is its most widespread use [14].

The future of HIFU is indeed bright as more areas of its application are being discovered. Current research into the expansion of the use of HIFU in the management of oncological disease shows that it is a safe and effective non-invasive method of targeting tumours [15]. Studies have shown positive results when investigating tumour shrinkage, patient safety, post-procedure complications. HIFU, both MRI and Ultrasound guided, have a variety of future applications, especially in patients that meet the suitable criteria [15].

WORKING PRINCIPLE

High Intensity Focused Ultrasound utilizes ultrasound waves to raise the temperature of tissue above 65 degrees Celsius. The High Intensity ultrasound wave is directed towards a very specific anatomical site at particular depth. This allows the sparing of surrounding tissues and is a precise method for targeting tissues [16]. HIFU is similar to diagnostic ultrasound in principle, the only difference is that HIFU, as its name implies, functions by generating

ultrasound waves at intensities that are several magnitudes higher than imaging/diagnostic ultrasound [17]. The therapeutic range of HIFU is between 100 W/cm² and 10,000 W/cm² [18]. The main objective is to utilize the thermal and mechanical effects of high intensity ultrasound waves to achieve tissue ablation through coagulative necrosis. Cell destruction through coagulative necrosis is achieved when the temperature of tissue is raised to 60 degrees celsius for 1 second. As a safety measure, the exposure does not last longer than 1 second [17].

The ultrasound wave is focussed to achieve the desired effects on tissue. HIFU transducers are designed to focus the beam through spherical, concave or sometimes flat surfaces of transducers. In some cases, acoustic lens are used for adjusting the ultrasound beam so that it is focused at a specific focal point (Fig 1). Aside from the thermal effects of HIFU, the mechanical effects of ultrasound are utilized to achieve acoustic cavitation, which aid in tissue destruction [18].

There are two major imaging modalities used in HIFU, MRI and ultrasound. Both modalities are utilized for targeting tissue, intraoperative beam localization and therapeutic monitoring post procedure [18]. MRI allows for high contrast imaging with precise spatial assessment but is limited because it does not allow real-time monitoring like ultrasound. The advantage of the MRI over ultrasound in HIFU is that it is the only modality to allow real time thermometric assessment, however, research into new technology is underway to expand the capabilities of ultrasound in this regard [18].

Contrast agents play an important role in HIFU, and the use of contrast agents in ultrasound is termed Contrast Enhanced Ultrasound (CEUS). Their application is important for the diagnosis of tumours, as a guiding tool in HIFU therapy and for assessing ablation during and after the procedure. Contrast agents form microbubbles in blood vessels which create acoustic changes in tissues, allowing for an increase in effectiveness of tumour ablation. A number of contrast agents are used, such as SonoVue (sulfur hexafluoride) and Sonazoid (Perfluorobutane), among others. A number of studies have demonstrated their safety and effectiveness [19-21]. We demonstrate a case of a patient with uterine fibroids treated USgHIFU (Fig 2A-C).

The HIFU JC system is an ultrasound guided device that allows real-time imaging during tumour ablation procedures. It consists of two transducer heads - one is diagnostic and operates at 3.5 MHz, and the other is the therapeutic, which operates at 1.6 MHz. This system lets precise targeting of tissue while monitoring patient movement and possible displacement of target point [23]. Coagulative necrosis presents as areas of hyperechoic spots (Fig. 2C). The patient is positioned on the HIFU table under which a special container filled with degassed water is placed. Degassed water is an efficient conductor of ultrasound waves and also fulfills the purpose of cooling the skin and subcutaneous tissue, preventing skin burns [22, 23].

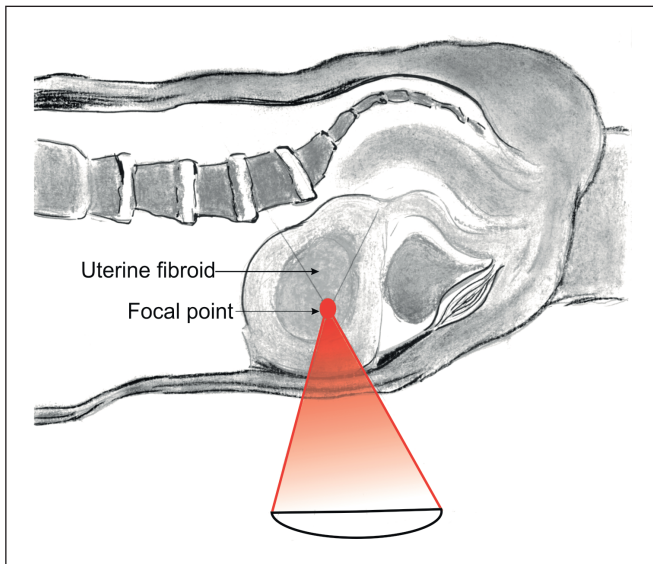


Fig. 1. Principle of HIFU ablation.

PATIENT SELECTION CRITERIA

Patient selection criteria is essential to the success of HIFU in the treatment of UF. The patient selection criteria was based on a prospective study establishing a clinical protocol for the use of HIFU in the treatment of UF, conducted by our institution (Table. I) [24]. Contrast enhanced MRI of the pelvis was used as the primary imaging modality [24].

Excluding criteria for HIFU ablation are these which may indicate a malignancy - low hemoglobin levels (less than 70 g/L), LDH exceeding 500 U/L, rapidly growing fibroid and heteroinsive zones with liquid-equivalent MRI zones.

Preoperative preparation of a patient before HIFU ablation of a myoma:

1. Taking a medical history
2. Examination by an internist
3. Gynecological examination
4. MRI examination
5. Three-day special diet before FUS

6. Preparation of the gastrointestinal tract - taking a cleanser the day before the procedure
7. Ablation is performed 3-5 days after menstruation

PROCEDURE

MAIN PROCEDURE

Preoperatively, the patient is catheterized for controlling the amount of urine in the bladder and for providing intracorporeal cooling. If required, serum at a certain temperature is introduced in the catheter and this allows for intraoperative temperature control. The patient is then sedated enough to achieve muscle relaxation but also to ensure effective communication with the operative team. The patient can communicate any discomfort or pain and the operative team can respond accordingly [24].

The patient is placed in the prone position on the operating table and the abdomen is immersed in a vat of degassed water, the temperature of which can be regulated by the operating team. A water balloon is placed between the transducer and the abdomen. The water balloon serves two functions, the first is to clear the intestinal loops around the tumour and to fixate the tumour in the acoustic pathway, the second function is to act as an acoustic lens to better focus the ultrasound beam.

Intraoperative imaging is then initiated to visualize the pelvis. After locating the tumour, the coordinates of the tumour are entered into the Model JC system for ablation planning. Sectional planes of the myoma are prepared digitally for ablation. Thermal ablation begins at low energy, usually at 50W and the patient response is noted. The energy of the HIFU beam is increased gradually till a maximum energy of 400 W. Diagnostic imaging allows for real-time visualization of the response of the tumour to ablative therapy. The presence of an hyperechogenic area indicates that coagulative necrosis has been achieved (Fig. 2C) [24].

Protocols for patient safety

Safety protocols are central to HIFU, and broadly revolve around temperature control. Factors that affect the safety

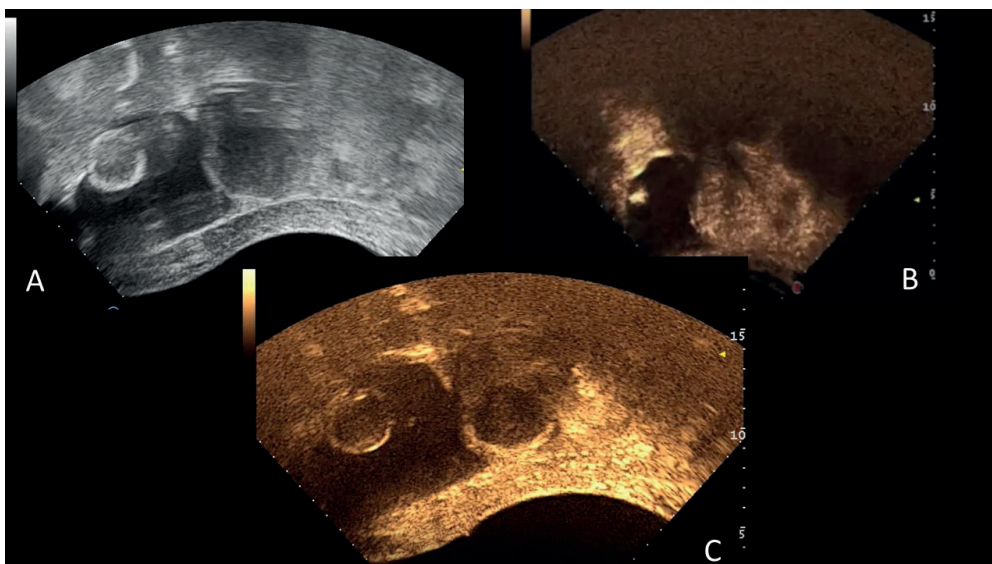


Fig. 2. An own case of a patient with uterine fibroids treated by ultrasound guided HIFU: A) a normal ultrasound image of the uterus fibroid and on the left, the urine-filled bladder and urethral catheter balloon are observed; B) 1.5 ml of Sonovue ultrasound contrast agent is injected and typical image of CEUS is observed - a well-supplied myoma is filled with "glowing" microbubbles that are present in the general circulation; C) an ablated fibroid with coagulative necrosis, as the microbubbles from the second dose of 1.5 ml Sonovue fill and enter only the uterine wall and other organs - the fibroid remains a "black" hole, which proves that the procedure was successful in achieving tumour ablation

Table I. Indicatoins and contraindications for HIFU.

Indications	Contraindications
Clinical diagnosed fibroid	Pregnancy
Dysmenorrhea, secondary anemia, sterility, abnormal menstrual cycle	Endometriosis
The diameter of the node should be greater than 2 cm when it is on the anterior uterine wall and larger than 4 cm when it is located in the area of the posterior uterine wall	Cervical fibroids
Consent to HIFU	Submucosal/Subserosal fibroid with pedicle
Consent for uterus preservation and refusal of hysterectomy	Diameter of posterior wall fibroid less than 3.5 cm
Low and moderate grade vascularization of fibroid nodules (T2 hypo to medium intense)	Large postoperative abdominal scar or foreign body implants in the acoustic path
Less than 4 fibroid nodules	Radiation dose over 45 Gy in the pelvis
BMI lower than 30	Hypovascular fibroid nodule
Distance between skin and the farthest depth of fibroid on the posterior wall is less than 9 cm.	Subcutaneous fat thickness greater than 10 cm
	Malignancy such as uterine sarcoma, endometrial cancer etc

limitations are the vascularization of the tumour, patient characteristics such as age, comorbidities and general status. The energy of the HIFU beam does not exceed 400 W and usually ranges between 200 to 400 W. An interval of 3 seconds is maintained between each successive ultrasound shot and it is not recommended to use a maximum power of 400 W. For every 300 seconds of ablation time, the procedure is paused for 5 to 7 minutes. This allows for minimizing the risk of thermal and cavitation injuries to tissues. Maintaining a distance between the mucous membrane and ultrasound rays, and between the sacrum and the ultrasound rays is central to patient safety. The procedure is stopped if tumour ablation is achieved or if the patient expresses discomfort or pain.

SonoVue dosage in HIFU is 25 mg dissolved in 5 ml of saline, with 1.5 ml being administered before, during and after HIFU ablation. After each dose, 10 ml of saline is administered intravenously [25, 26]. When using Sonazoid, it is necessary to make one application for 30 minutes before the onset of ablation and it provides the presence of contrast for about 60 minutes in the bloodstream. Reconstitution is performed according to the manufacturer's instructions [25]. After the procedure, the patient must remain in the prone position for 10 minutes to allow the cooling of abdominal skin.

POST PROCEDURE STATUS

OUTCOMES

The clinical outcomes of patients who underwent HIFU are comparable to other therapeutic interventions. HIFU is a safe measure and can be argued to be a suitable alternative to other therapeutic methods [24, 27]. Although meta-analyses comparing Uterine Artery Embolization (UAE) and HIFU have been conducted, they are limited by the low number of studies included and also do not address the outcomes as they

relates to pregnancies, both successful or otherwise [28]. Patients who have undergone HIFU report lower post operative pain, fever, and return to their daily lives sooner than those who undergo invasive surgical procedures like hysterectomies [24]. The comparison between UsgHIFU and laparoscopic myomectomy shows a comparability in improvement of QoL inwomen and fewer significant clinical complications and adverse events and with faster recovery [29].

HIFU as a treatment for uterine fibroids is dependent on strict adherence to the Patient Selection criteria and deviations, such as variations in tumour size, increase reintervention rates [30]. Recovery time in patients who have underwent HIFU is lower and the number of pregnancies is also higher than those who underwent UAE or other surgical interventions [31]. The most significant advantage of HIFU was the lower incidence of postoperative pain and discomfort, these adverse outcomes did not present with permanent sequelae or death [28]. Significant improvements in the Quality of Life after 36 months indicated that patients who underwent HIFU reported comparable or higher Quality of Life [31].

The introduction of FUS as an opportunity for leading choice in patients with fibroid disease requires research and greater experience among European patients, as well as the comparison of the method with other surgical approaches. This was conducted in Oxford - IDEAL non-randomized prospective study. It involves 2411 patients with symptomatic fibroids, and they have the right to choose the treatment approach. All patients are premenopausal, the uterus is enlarged at least as much as 10 gestational weeks, with no more than 3 myomas, and the maximum allowable size of a single fibroid is 10 cm. 1353 women chose HIFU, 586 requested myomectomy and 472 underwent hysterectomy. The study proves that both quality of life and symptoms have improved significantly faster in the HIFU group. Significant side effects occurred in 0.2% of the HIFU group and in 12.6% of patients who underwent surgery. The mean time to hospitalization was 4/8/10 days for the three groups, respectively. At 6-month follow-up, the pregnancies

occurred were 7/1/0, respectively, and at 12-month follow-up 21/3/0. After 12 months, there was a need for re-intervention at 14/0/0, respectively, which is 1% for the HIFU group and 0 for those who underwent surgical treatment. The conclusion proves similar effectiveness in improving the quality of life and the response to symptoms, as in the HIFU group there is a significant short hospital stay, as well as early return to work and a small percentage of side effects [32].

COMPLICATIONS

The most common adverse effects in HIFU are postoperative pain, discomfort and mild skin burns. However, none of these adverse effects have presented with long term sequelae or death [28]. The need for reintervention is another possible complication, however, reintervention rates are consistently low in HIFU patients [27], and this can be avoided as long as patient selection criteria is adhered to [31]. Over a period of 4 years (between 2011-2015), 10,310 patients were followed up in China to investigate complications. Retrospectively, 4136 side effects were identified in 2367 patients (23%). They most often complained of pain in the lower abdomen 21.9% (2253/10310), followed by the presence of minimal vaginal discharge 11% (1136 patients), 6.9% reported sacro-coccygeal pain. The complaints subsided without treatment for about a week. Thirteen patients (0.1%) reported tingling or pain in diseased limbs that lasted between 2 weeks and 2 months. Among the more serious complications were skin burns in 21 women (0.2%) and colon damage in 2 of the patients (0.02%). The burns were of 2nd and 3rd degree and were found mainly in women with scars on the lower abdomen. It was found that patients who had perforation of the intestine did not follow the protocol for cleansing the stomach - the intestinal tract before the intervention, as well as the use of high energy in ablation [27].

CONCLUSION

The use of UsgHIFU as a therapeutic intervention for uterine fibroids is an area of promising results. Its primary advantage is that it can be performed non-invasively and without the need for general anesthesia. Clinical outcomes for HIFU in general are promising in the treatment of uterine fibroids, and should be recommended for patients who fit the selection criteria. The Patient Selection Criteria is essential to positive outcomes of UsgHIFU and care should be taken to ensure that patients meet this criteria for optimal outcomes. Further research comparing the various modalities of therapeutic interventions should be conducted to suggest standardized protocols of care.

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ORCID and contributionship:

Yoana Ivanova - 0000-0002-2215-9202 ^{A,D}
 Dobromir Dimitrov - 0000-0003-3313-1093 ^{E-F}
 Kameliya Dimitrova - 0000-0003-1418-6179 ^{B-C}
 Aparajeya Shanker - 0000-0001-6305-8152 ^{B-C}
 Angel Yordanov - 0000-0002-7719-382X ^{E-F}

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CORRESPONDING AUTHOR

Angel Yordanov

Department of Gynecologic Oncology,
 Medical University Plevan, Plevan, Bulgaria
 e-mail: angel.jordanov@gmail.com

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CASE STUDY

PRINCIPLES OF DIAGNOSIS AND TREATMENT OF ASKIN'S TUMOR IN CHILDREN: CASE REPORT

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Tatiana G. Korol, Serhii S. Blazhko, Hennadii M. Rudenko, Kateryna Khromykh

NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

The aim of the study was to show principles of diagnosis and treatment of Askin's tumor in children. Diagnostic procedures include physical examination, chest X-ray, CT scan and PET CT, morphological, histological and immunohistochemical examinations, cytogenetic study. Primitive neuroectodermal tumors belong to the group of low differentiated, overly aggressive neoplasms, originating from cells of the parasympathetic autonomic nervous system. Patient F., 9 years old, first consulted by pediatric oncologist in 2014 with complaints of volume formation in the chest on the right side which progressively increases. Diagnosis: PNET (primitive neuroectodermal tumor) of the soft tissues of the chest on the right side in the 4th intercostal space along the midclavicular line T2aNOMO, stage 2a, standard risk group. We've shown results of diagnostic process, treatment and its result in our patient. Patients who have received combination therapy, including chemotherapy, surgical removal of the tumor and radiation therapy, have better prognostic results. However, relapses often occur that require more aggressive treatment with high-dose chemotherapy, monoclonal antibodies, and bone marrow transplantation.

KEY WORDS: Askin's tumor, children

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INTRODUCTION

Primitive neuroectodermal tumors (PNET) belong to the group of low differentiated, overly aggressive neoplasms, originating from cells of the parasympathetic autonomic nervous system. The incidence is about 4-17% of all soft tissue tumors in childhood. By origin, they are divided into peripheral PNET (pPNET), identical to Ewing's sarcoma, and central PNET (cPNET), which requires differential diagnosis with medulloblastoma, epindymoma, pinealoma, rhabdomyosarcoma, and neuroblastoma with primary brain damage [1]. In 1979, Askin first described 20 cases of PNET growing from the soft tissues of the thoracic area in children and adolescents, since then they have been called «Askin's tumor». These tumors in histological, immunohistochemical, cytogenetic and phenotypic similarity belong to the Ewing's sarcoma family, but are extremely rare [2,3].

Diagnostic procedures include physical examination, chest X-ray, CT scan and PET CT, morphological, histological and immunohistochemical examinations, cytogenetic study. This tumor on palpation is slightly denser than the soft tissues of the chest wall, often there is destruction of the ribs, pleural effusion, which is visualized by chest radiography. However, it is necessary to perform CT scan of the thoracic and abdominal cavities and MRI of the chest wall to determine the size of the tumor, possible invasion of the lungs and the presence of distant metastases [3]. 26-28% of children have initially distant metastases and in about 30% of patients metastasis to the bone marrow is confirmed [4-6]. PET CT plays an equally important role in the detection of metabolically active tumors at the

stage of primary diagnosis and to assess the response to treatment [4]. Morphologically, Askin's tumor is gray-white with necrotic, cystic and hemorrhagic areas in section. Histological examination reveals monomorphic small blue round cells, which probably originate from the neural crest [5]. Cytogenetic study in this case has an important diagnostic value. Thus, in 85% of cases there is a mutual translocation of t(11:22) (q24: q12) with the gene EWS-FLI-1. Checking presence of proto-oncogenes such as n-myc, c-myb, c-ets-1 and tumor markers (NSE, LDH) can be additional diagnostic criteria [7]. There are no PNET-specific markers, but CD99 is detected in most patients on immunohistochemical examination of tumor cell surfaces [8].

This tumor has rapid aggressive growth, frequent metastasis and progress during treatment. The prognosis is very poor. 2-year survival from diagnosis is less than 40%. Therefore, it is necessary to study new treatment strategies (chemotherapy, immunotherapy) in combination with autologous hematopoietic stem cell transplantation (auto THC) in order to improve the quality of life and survival of patients [9]. In their studies, Lascar S. and co-authors have shown that the best results are achieved with a combination of systemic (non-adjuvant and adjuvant) chemotherapy, surgical treatment and radiation therapy [10]. The first line of chemotherapy includes drugs such as vincristine, doxorubicin, cyclophosphamide, etoposide and ifosfamide [11, 12]. Frequent relapses and progression of the disease after first-line therapy required further research and the search for new treatment options that include cyclophos-

Markers	Present/absent
Monoclonal Mouse Anti-Human CD99. MIC 2 Gene Product Ewing's Sarcoma Marker Clone 12B7 (Dako IS57)	"+"
Rabbit Monoclonal Antibody to Fli-1 (DBS RMPD025)	"+"
Monoclonal Mouse AntiHuman Cytokeratin Clone AE1/AE3 (Dako M3515)	"+"
Monoclonal Mouse Antibody to Human Leukocyte Common Antigen CD45	"_"
Monoclonal Mouse Anti- Human Neuron Specific Enolase Clone	"_"
Monoclonal Mouse Anti-Human CD56	"_"
Polyclonal Rabbit Anti-S100 (Dako Z0311)	"_"
Monoclonal Mouse Anti-Myogenin Clone F5D (Dako IS067)	"_"
Monoclonal Mouse Antibody to MyoD1 Clone 5.2F (DBS PDM120)	"_"
Monoclonal Mouse Anti-Human Desmin Clone D33 (Dako M0760)	"_"
Monoclonal Mouse Anti-Human CD34 Class II Clone QBEnd 10 (Dako IS32)	"_"
Conclusion: according to the results of morphological and immunohistochemical studies, the phenotype characteristic of primitive neuroectodermal tumor (PNET).	

Table II. The European pediatric Soft Tissue Sarcoma Study Group (EpSSG) RMS 2005 study high-risk localized rhabdomyosarcoma[illegible]

phamide and topotecan, irinotecan and temozolomide in combination with high doses of ifosfamide. Nowadays, there are investigations for using of monoclonal antibodies, such as bevacizumab (Avastin) and sunitinib [13]. The long-term prognosis for Askin's tumor depends on the initial size, the presence of metastases, the level of LDH and combination therapy, namely surgery, chemotherapy and radiation therapy [14].

CASE REPORT

Patient F, 9 years old, first consulted by pediatric oncologist in 2014 with complaints of volume formation in the chest on the right side which progressively increases. He was hospitalized for additional examination and diagnosis. Results of the tests were performed:

- ultrasound of soft tissues (14.01.2014): tumor in the 2-4 ribs region, size $86 \times 26 \times 55$ mm, with its own blood flow.
- CT scan of the chest and abdominal cavity with IV contrasting (15.01.2014): soft tissue tumor formation $81 \times 58 \times 25$ mm (tumor volume is 62 cm^3) in the thick-

ness of the chest wall; distant metastases to the chest, abdomen and retroperitoneal space were not detected (fig. 1).

Trepan - tumor biopsy, trepan bone marrow biopsy from 3 points and liquid bone marrow (20.01.2014). Tumor histology showed PNEP. Bone marrow without signs of damage. The result of immunohistochemical investigation present in table I.

Diagnosis: PNEP (primitive neuroectodermal tumor) of the soft tissues of the chest on the right side in the 4th intercostal space along the midclavicular line T_{2a}N₀M₀, stage 2a, standard risk group.

The child began treatment according to the CWS 2006 protocol on February 10, 2014.

The central venous port system was set up on March 4, 2014. After the 2nd block of chemotherapy, the tumor was not clinically determined. Control CT scan of the chest with IV contrast was performed on April 17, 2014: Residual tumor is not determined. Continuation of treatment according to the protocol on the line «complete response».

Treatment included 9 blocks of chemotherapy and radiation therapy of 40 Gr per pre-therapeutic volume of

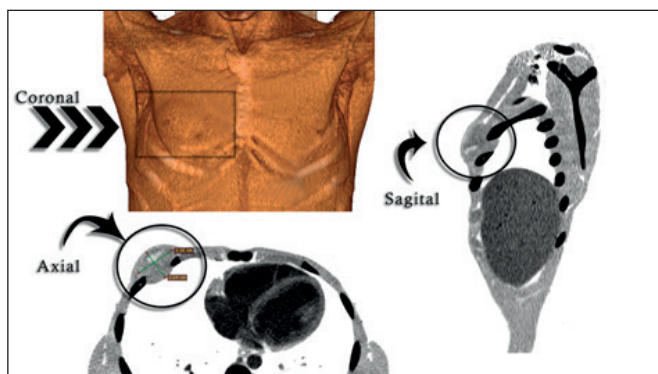


Fig. 1. CT scan of the chest and abdominal cavity shows soft tissue tumor

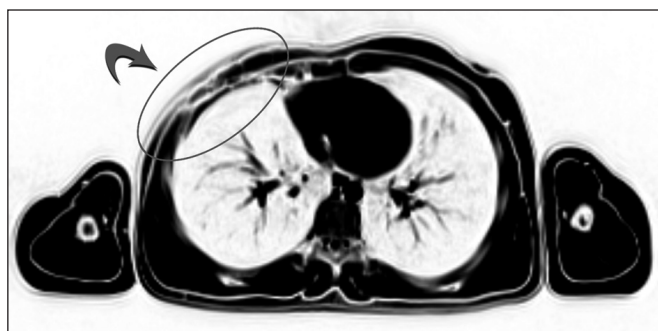


Fig. 2. Post operating seroma of the right sectoral area with perifocal fibrous post operating changes on CT

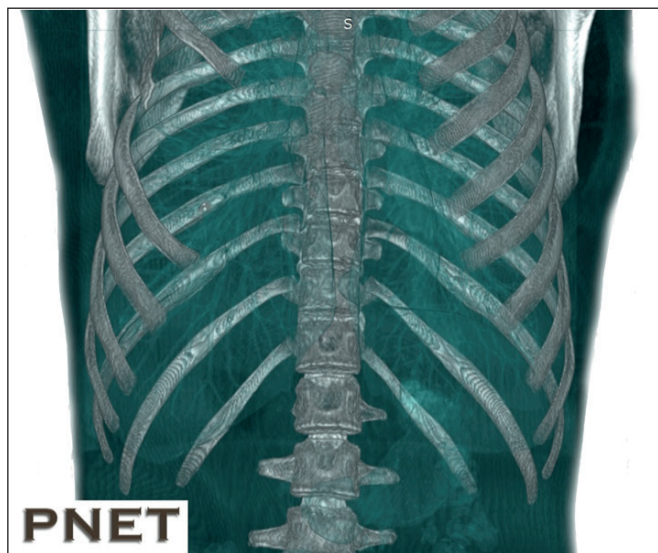


Fig. 3. Control contrast chest CT scan shows remission

the tumor (anterior surface of the chest on the right side). After this treatment child was include into the observation group (3rd clinical groups). Regularly passed routine examinations, including contrast CT scan of the chest and abdominal cavity, contrast MRI of soft tissues of the chest every 3 months. According to the results of examinations, no pathological changes were detected.

On December 6, 2016 (2 years of remission) child came for an examination with complaints of pain and the presence of a voluminous formation of the chest on the right side along the midclavicular line, and increase body

temperature to 37.1°C. Patient was hospitalized for further examination with diagnosis: the first recurrence of PNET?

Surveys were conducted:

Scintigraphy with radiopharmaceutical drug (9.12.2016): accumulation of the drug in the area of the head and upper third of the left femur - 280%.

Contrast CT scan of the chest (12.12.2016): additional volume formation of the anterior chest wall on the right side at the level of the anterior segments of 4 - 5 ribs on the right along the mid-clavicular line.

Surgical intervention was performed (15.12.2016): open biopsy of the tumor, trepan – biopsy of the bone marrow from the iliac crests and collected liquid bone marrow.

Extract from the investigation protocol: access over the three-dimensional formation along the midclavicular line in 4 intercostal spaces up to 3 cm, soft tissues are dissected in layers, revealed: two formations, one of which is covered with a hard fibrous capsule, the other next to the capsule - excisional biopsy of both formations.

The results of histological examination:

Tumor: growth of primitive neuroectodermal tumor (both samples)

Histological examination of the bone marrow: signs of depletion of all hematopoietic sprouts and fibrosis

Cytological examination of bone marrow: cellularity is slightly reduced, normoblastic type of hematopoiesis. When counting at low magnification about - 3 megakaryocytes.

Diagnosis: PNET (primitive neuroectodermal tumor) of the soft tissues of the chest on the right side in the 4th intercostal space along the midclavicular line $T_{2a}N_0M_0$, stage - 2a, standard risk group. The first relapse.

The 2nd line of chemotherapy was started (table II). 2 blocks of therapy with etoposide, carboplatin, cyclophosphamide were performed as a mobilization course for the purpose of collection of autologous peripheral blood stem cells. A collection of material for autologous bone marrow transplantation was performed, but was unsuccessful due to severe aplasia.

The medical commission decided to carry out radical surgery, given the ineffectiveness of chemotherapy. Radical removal of the tumor with resection of IV and V ribs was performed on March 7, 2017. Plasticity of defect by a propylene grid, drainage of a pleural cavity and soft tissues were made. Post operating period gone without complications.

Histology: the growth of PNET. After the operation child received 2 more chemotherapy blocks. After this treatment child had a remission and was include into the observation group.

20.09.2018 child came for routine examination with a suspicion of relapse. Surgical intervention was made (26.09.18): removal of a tumor on the right side of the chest.

There were 6 samples of the tumor resection edge:

№1 – the centers of growth of a malignant undifferentiated tumor among fibromuscular tissue;

№2 – fibrous - adipose tissue without signs of malignant growth;

№3 – fibrous - adipose tissue with hemorrhages, slight inflammation;

№4 – muscle tissue without signs of malignant growth;

№5 – muscle tissue without signs of malignant growth;

№6 – fibrous - adipose tissue without signs of malignant growth.

Trepan - biopsy of the bone marrow: the bone marrow is represented by red and yellow in a ratio of 1: 1. Red normocellular represented by all three sprouts with a predominance of granulocyte.

Morphological examination of the bone marrow (26.09.18): the cellularity of the bone marrow is reduced. Normoblastic type of hematopoiesis. 7 megakaryocytes were found at low magnification in a smear.

Contrast CT scan of the chest and abdominal cavity (05.10.18): picture of the condition after resection of the 4th - 5th ribs and soft tissues on the right side of the chest wall, accumulation of fluid in the soft tissues of the anterior chest wall on the right - surgical changes. Pneumofibrosis on the right D4 and D5. Single stable nodules D6,8. MRI of the chest with intravenous contrast (08.10.18): condition after combined treatment of PNET of the right sector. Post operating seroma of the right sectoral area with perifocal fibrous post operating changes (Fig. 2)

After surgery child received 3 courses of chemotherapy (Irinotecan - Temodal).

PET/CT scan (19.02.2019): no signs of pathological radioactive pharmaceutical drug accumulation were detected. Fixation of radioactive pharmaceutical drug in a scar in soft tissues of a chest wall is more characteristic to after therapeutic changes as there are no convincing data for additional tumor with local accumulation of radioactive pharmaceutical drug. There is no convincing evidence for the presence of radioactive pharmaceutical drug active malignant process. Control of contrast CT scan of the chest (06.09.2019)(fig. 3). Remission continues.

PNET belongs to a group of highly aggressive malignant neoplasms, the histological substrate of which are small undifferentiated neuroectodermal cells [13]. Askin's tumor belongs to the group of peripheral PNET with primary lesions of the chest. It is often localized in the paravertebral areas [15]. Small cell sarcomas are a heterogeneous group of malignant neoplasms that remain diagnostically complex due to similar morphological and immunohistochemical characteristics. They are more often diagnosed in adolescents and young adults and have a prognostic course [16].

For this tumor, there are typical morphological features such as the presence of small cells with Homer-Wright rosettes. In immunohistochemical study positive CD 99. The presence of translocation t(11;22)(q24,q12) and proto-oncogenes c-myc, n-myc, c-ets-1, which are important diagnostic criteria [7]. A significant increase in the level of LDH, relative to reference values, is considered a poor prognostic factor [19]. Usually this neoplasm recurs locally, but separate metastases can be detected [17]. This tumor is very rare and too aggressive, so the searching for new treatments that will improve the long-term prognosis is going all the time. Combined treatment involving surgical removal of the tumor with extensive resection of the

edges in combination with chemotherapy (ifosfamide, vincristine, etoposide, D-actinomycin, doxorubicin) and radiation therapy has been shown to give better prognostic results and reduce the frequency of 3 recurrences [18].

CONCLUSIONS

Patients who have received combination therapy, including chemotherapy, surgical removal of the tumor and radiation therapy, have better prognostic results. However, relapses often occur that require more aggressive treatment with high-dose chemotherapy, monoclonal antibodies, and bone marrow transplantation.

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ORCID and contributionship:

Tatiana G. Korol: 0000-0002-7240-6056 ^{A,E}

Serhii S. Blazhko: 0000-0003-4891-5886 ^B

Hennadii M. Rudenko: 0000-0003-2799-1900 ^F

Kateryna Khromykh: 0000-0001-7241-5190 ^D

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CORRESPONDING AUTHOR

Kateryna Khromykh

National Pirogov Memorial Medical University

56 Pirogova st., 21018 Vinnitsia, Ukraine

tel: +380634009099

e-mail: kate_khromykh@yahoo.com

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