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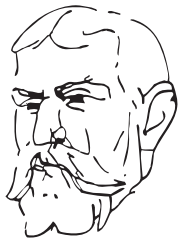

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CONTENTS

ORIGINAL ARTICLES

REVIEW ARTICLES

CASE STUDIES

ORIGINAL ARTICLE

OPTIMIZED METHOD OF BOVINE PERICARDIUM DECELLULARIZATION FOR TISSUE ENGINEERING

DOI: 10.36740/WLek202104101

Nataliia V. Shchotkina[1,2], Anatoliy A. Sokol[1], Oleksandr Yu. Galkin[2], Glib I. Yemets[1], Liudmyla V. Dolinchuk[2], Nadiya M. Rudenko[1], Iliia M. Yemets[1]

[1]CENTER FOR PEDIATRIC CARDIOLOGY AND CARDIO SURGERY, KYIV, UKRAINE

[2]NATIONAL TECHNICAL UNIVERSITY OF UKRAINE "IGOR SIKORSKY KYIV POLYTECHNIC INSTITUTE", KYIV, UKRAINE

ABSTRACT

The aim: To investigate the effectiveness of using low concentrations of sodium dodecyl sulfate (SDS) and cross-linking with EDC/NHS in the decellularization process to create a potential bioimplant for cardiac surgery.

Materials and methods: Pericardial sacs were derived from 12-18 months bulls. Tissue decellularization was performed by using 0.1% SDS with the following EDC/NHS cross-linking. The experiment included standard histological, microscopic, molecular genetic and biomechanical methods. Scaffold was tested in vitro for cytotoxicity and biocompatibility.

Results: A high degree of extracellular decellularized matrix purification from cells and their components was shown. Structure-function properties remained similar to those or even improved after the decellularization. During prolonged contact of BP with human fibroblasts, no cytotoxic effect was observed. The biointegration of the scaffold in laboratory animals tissues was noted confirming the potential possibility of the implant use in cardiac surgery.

Conclusions: Decellularization of BP by 0.1 % SDS with NHS/EDC cross-linking is promising in manufacturing of the tissue-engineered materials in cardiac surgery.

KEY WORDS: tissue engineering, cardio implant, xenograft, bovine pericardium

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INTRODUCTION

Biotechnological progress is closely related to the development and introduction into clinical practice of biological materials from xenografts. Decellularized tissues are widely used in the reconstructive surgery. For more than five decades, xenografts have been widely used both in pediatric and adult heart surgery and blood vessels for the correction of acquired and congenital heart defects [1].

Nowadays, one of the most common types of bioimplants is the pericardial patch (scafold) – a collagen matrix of calf pericardium which is either purified from cells by decellularization or structurally stabilized with glutaraldehyde. For the decelularization of pericardium high efficiency of detergents, including ionic detergent sodium dodecyl sulfate (SDS) has been proven [2]. However, these agents should be used at low concentrations to reduce alterations in the ultrastructure of the extracorporeal matrix and maintain glycosaminoglycan (GAG) concentrations [3]. Also, there are some difficulties in using glutaraldehyde, which purifies the tissue and is used to form new covalent bonds between two polymer chains to strengthen the structure of the matrix. Incomplete elimination of residual glutaraldehyde prior scaffold implantation can cause cytotoxic effects on human cells and late calcification of scaffold *in vivo* [4].

Fixedy by glutaraldehyde pericardial tissue is commonly used for commercially available xenograft valve implants and has proven durability but lacks the capability for remodelling and growing. Decellularized bovine pericardial tissue is promising to outperform fixed tissue and able to remodel [5]. Thus, the task arose to find a way to create an optimal bioimplant. In this work, it was proposed to test an optimized technique for decellularization and glutaraldehyde-free stabilization of the bovine pericardium matrix.

THE AIM

The aim of this study was to investigate the effectiveness of using low concentrations of SDS in the decellularization and cross-linking with EDC/NHS for fixation to create a potential bioimplant for cardiac surgery.

MATERIALS AND METHODS

THE PROCEDURE FOR TISSUE OBTAINING

The material for the study was the bovine pericardium (BP). The pericardial sac was extracted from outbred 12–18 month old bulls after slaughter at the "Antonivskii Meatplant Ltd.". The research was performed in accordance with the General Ethical Principles of Animal Experiments (Strasbourg, France, 1985) and Law of Ukraine No. 3447 – IV On Protection of Animals from Cruel Treatment (2006, edited in 2009). The biomaterial was transported to the laboratory for one hour in a sterile Hanks solution

in a container on ice. Pericardial sacs were dissected and non-fibrous components were removed. The tissue samples were cut in 40×40 mm. Samples were placed in distilled water in a volume of 1 000 ml and stirred continuously (70 rpm) for 3 hours at 4 °C.

GLUTARALDEHYDE-FREE DECELLULARIZATION OF THE BOVINE PERICARDIUM

BP samples were decellularized as follows: (1) osmotic shock was caused by placing samples into the sterile distilled water (5 pieces per 500 ml solution) at 4°C for 72 hours (200 rpm). Water was changed every 6 – 8 hours; (2) decellularization with 100 ml of 0.1% solution of SDS (Sigma-Aldrich, USA) with constant shaking (200 rpm) for 35 days at 24 °C; (3) washed with sterile NaCl solution for 7 days at 4 °C with constant stirring at 200 rpm; (4) stabilization and fixation in a solution of 70 % ethanol for 24 h at t 4 °C with constant stirring at 200 rpm; (5) washed with sterile NaCl solution for 24 h at 4 °C with constant stirring at 200 rpm; (6) cross-linking method [6] – EDC/NHS solution – MES (10 mM 1-Ethyl-3- (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 10 mM N-Hydroxysuccinimide) and MES solution (pH 5.6) (0.05 M 2–morpholinoethane sulfonic acid); (7) washed with sterile NaCl solution for 24 h at 4 °C with constant stirring at 200 rpm. Non decellularized pericardial tissues were chosen as control.

BIOMECHANICAL TESTING

The biomechanical properties of BP with a size of 20×40 mm were analyzed. A test machine (IMADA, MX2 — 110, Japan) was used to determine the maximal tensile strength (F_{max}).

DNA TEST

DNA extraction was performed with Easy Blood and Tissue DNA kit (Qiagen, Germany). 10–25 mg of tissue from external materials before decellularization or from decellularized samples, were briefly treated with proteinase K to quantify the total amount of nucleic acid (ng/mg of dry tissue) and the percentage of DNA removal after decellularization. Fluorescence measurements (photons per second) were performed at room temperature (23-24 °C) using a spectrofluorometer Qubit 3.0. The DNA detection limit was 0.2 ng/μg of nucleic acid.

CYTOTOXICITY IN VITRO TESTING

To determine cytotoxicity the matrix samples were cultured in a suspension of human fibroblasts. 5 fragments from each sample were cut from the obtained material (approximately 8–10 mm^2). Cells were inoculated by applying 150 μl of concentrated cell suspension (3×10^5) on moistened with standard growth medium (DMEM + 10% of serum) decellularized pericardial samples. Impregnation lasted 30 minutes. Cells were then transferred to a standard 6-well tissue culture plate and immersed in growth medium DMEM + 10% of serum. Cultivation was performed under standard conditions of 37 °C and 5% of CO_2 [7]. For histological examination, DEM samples were fixed in 10% buffered formalin embedded in paraffin. Sections were made (5 μm). Hematoxylin/eosin stained samples were obtained (light microscopy) [8]. Images were taken using an Olympus BX 51 light microscope (Tokyo, Japan).

BIOCOMPATIBILITY TEST *IN VIVO*

Ten 4-5 month old male Wistar rats weighing 190-230 g were kept in a pathogen free environment. Decellularized and sterilized bovine pericardium were implanted subcutaneously into the dorsal skin of rats and explanted after 3 months. The animals were divided into the following groups: Control Group – native bovine pericardium (n = 5), Group 1 – decellularized bovine pericardium with 0,1 % SDS and cross-linked bovine pericardium (n = 5).

The surgical field was shaved and cleansed with a 70% solution of ethyl alcohol. The surgery was performed in a sterile conditions. Intramuscular anesthesia was used with xylazine (Alfasan, the Netherlands) at a dose of 1 mg/kg body weight in combination with ketamine (Biolik, Ukraine) at a dose of 10 mg/kg. A 2 cm incision was performed in the dorsal skin and subcutaneous pockets were formed using a pointed spatula, separating the subcutaneous tissue from the muscular layer. Prepared 1 × 1 cm implants were placed in the pocket, which were fixed at the corners to the muscle tissue using „Catgut Chrome" („Golnit", Ukraine). Skin was sutured and treated with an antiseptic solution of 1% „Diamond Green" („Halychpharm", Ukraine). Implants were isolated for histological analysis after 3 months post-operation. Euthanasia was perfomed with ether.

HISTOLOGICAL ANALYSIS

Segments of non decellularized (n=10) and decellularized (n=10) pericardial tissues were fixed in10% neutral buffered formalin (Sigma-Aldrich, USA) for 1 h, embedded in paraffin, cut into 5-μm sections and stained with hematoxylin & eosin (H&E, Sigma Aldrich, USA), Congo red (Congo Red Kit, Sigma Aldrich, USA) and Masson's Trichrome stain (MT, Sigma Aldrich, USA). The stained samples were examined with Olympus BX 51 light microscope (Tokyo, Japan).

For scanning electron microscopy (SEM), samples of decellularized pericardium were dried by lyophilization at a vacuum depth of 30-50 Pa and a temperature of -50 ° C. (JFC-1100, Jeol, Tokyo, Japan). In order to eliminate the accumulation of surface charge during scanning with an electron beam in a microscope column, a thin layer of gold was applied to the dried samples by cathodic sputtering (JFC-1100, Jeol, Tokyo, Japan). The preparations were investigated in SEM JSM 6060 LA (Jeol, Tokyo, Japan) in the secondary electron mode at an electron acceleration voltage of 30 kV.

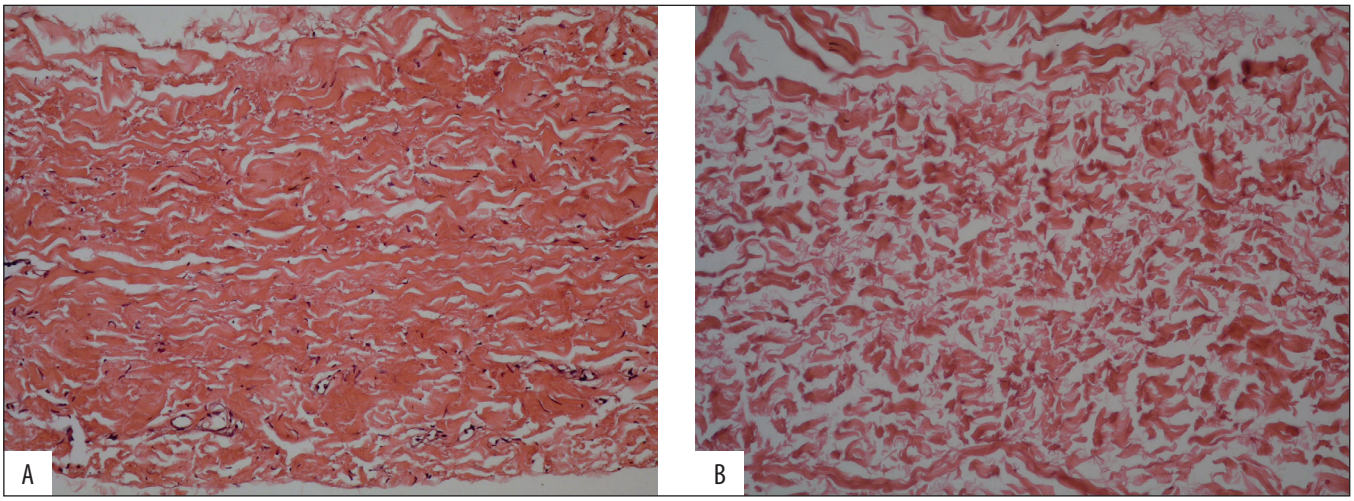

Fig. 1. Micrographs of histological sections of native and decellularized bovine pericardium; hematoxylin/eosin staining, light microscopy, x200. A – presence of visible violet cell nuclei in native bovine pericardium (control group); B – absence of visible cell nuclei in BP decellularized by 0.1% SDS (experimental group).

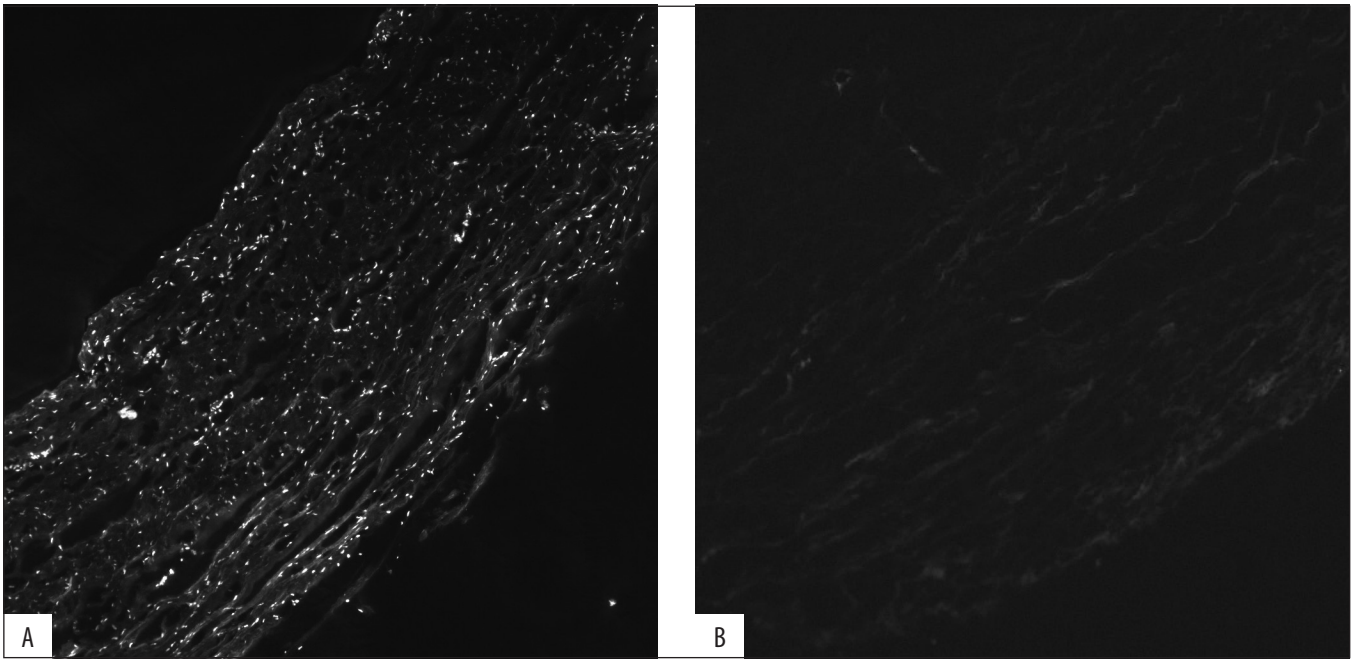

Fig. 2. Micrographs of histological sections of native and decellularized bovine pericardium; DAPI, fluorescence microscopy, x200. A – availability of nuclear fluorescence in native bovine pericardium (control group); B – absence of nuclear fluorescence in decellularized by 0.1% SDS solution BP (experimental group).

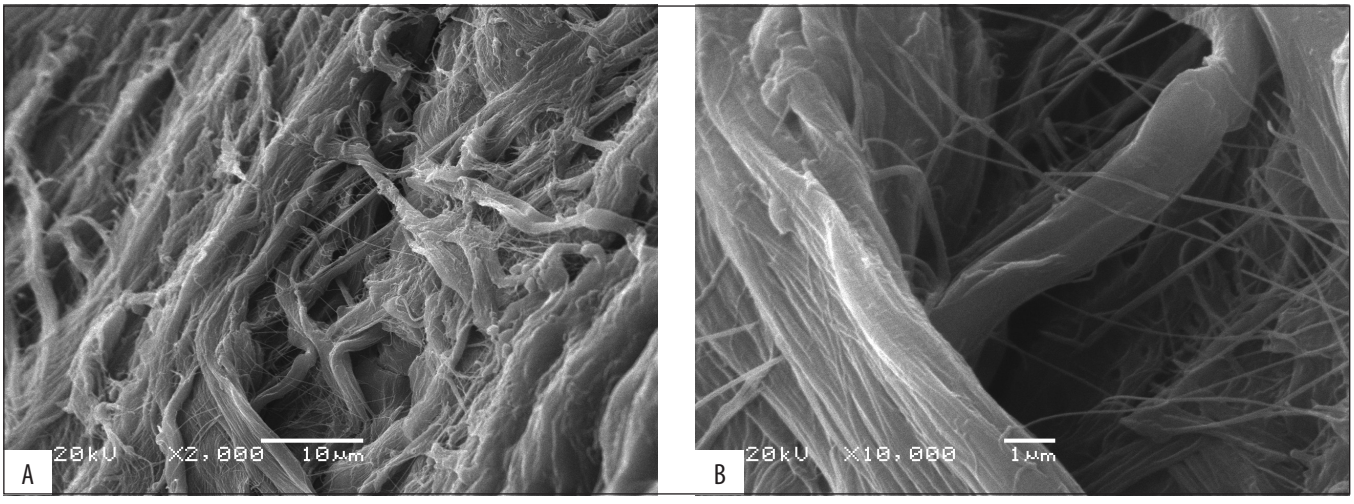

Fig. 3. Scanning electron micrographs. A — control group (native pericardium); B — experimental group (0.1% solution of SDS)

Table I. Comparative evaluation of native and decellularized bovine pericardium

Research	Control group	Experimental group
Average DNA content (ng/mg), $n = 5$	1436 ± 116,8	0,5 ± 0,45*
Longitudinal tissue testing Fmax, maximal tensile strength (kgf)	6,84 ± 0,69	9,55 ± 0,65*
Transverse tissue testing, Fmax, maximal tensile strength (kgf)	3,78 ± 0,36	4,56 ± 0,37*

* statistically significant compared with the control group ($P < 0.05$)

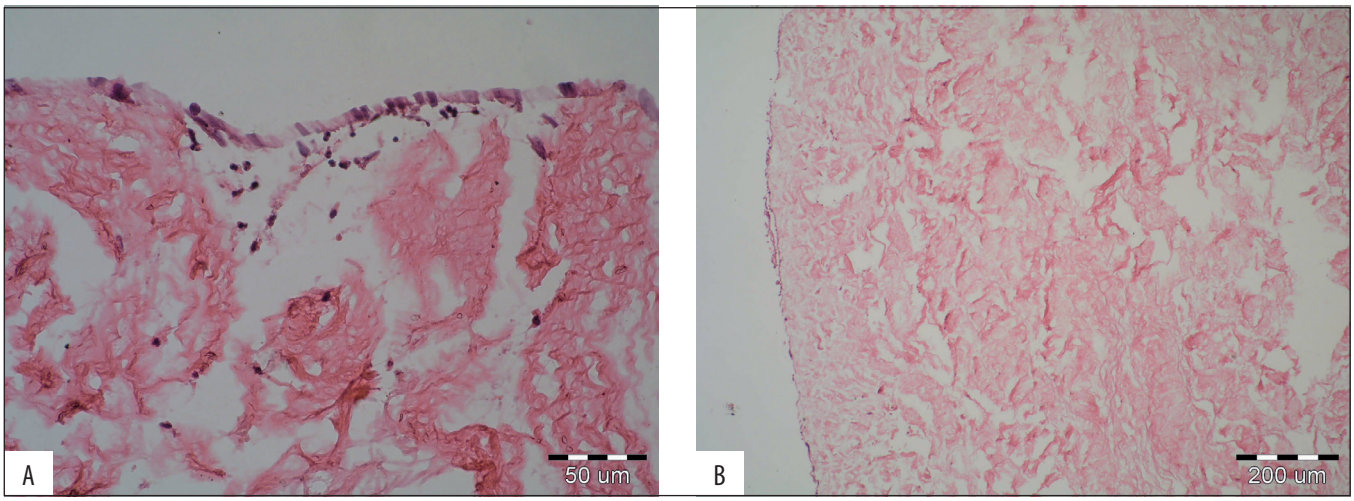

Fig. 4. Histological examination of experimental group (0.1% SDS) after 2 months of cultivation (Hematoxylin-eosin , light microscopy).

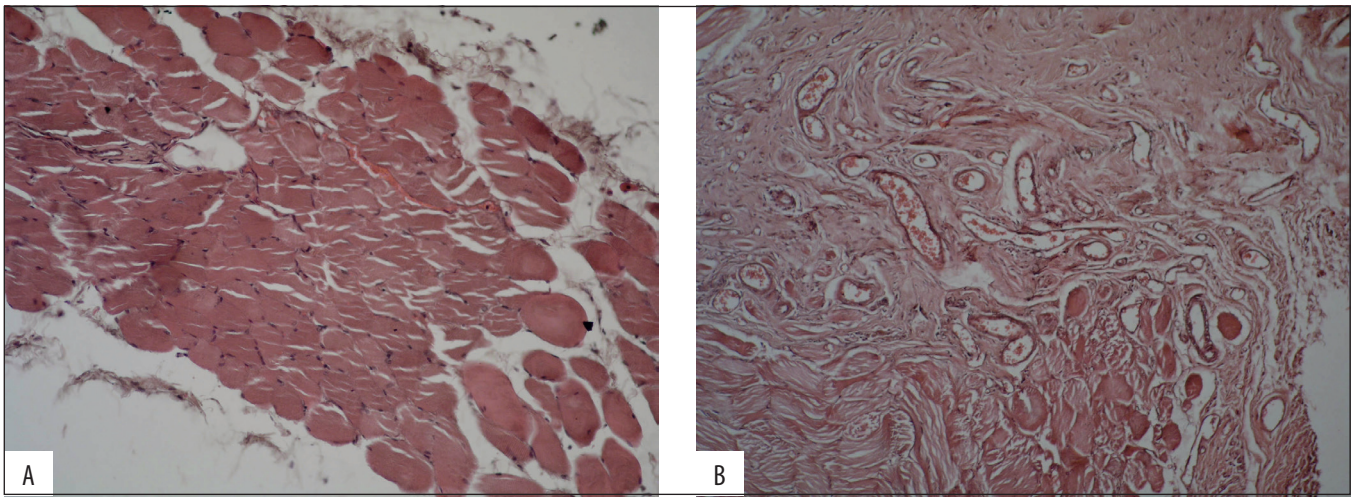

Fig. 5. Micrographs of histological sections in 3 months after subcutaneous implantation in rats; light microscopy, hematoxylin/eosin staining, x200. A – no visible implanted native pericardium, swelling of muscle tissue with leukocyte infiltration (black arrows); B – the formation of immature connective tissue over the entire surface of the graft

STATISTICAL ANALYSIS

The analysis of the research results was carried out using biostatistical methods [9]. For quantitative values, the normality distribution was analyzed using the Shapiro-Wilk test. The mean value (M) and standard deviation (± SD) were calculated. To estimate the mean value, its 95% confidence interval (95% CI) was calculated. For qualitative values, the frequency (%) and, if necessary, 95% CI were calculated. When comparing quantitative parameters in more than two groups, one-way ANOVA was used [9], posteriori comparisons were carried out using Scheffe Test (The distribution law did not differ from normal). To compare qualitative parameters, the chi-square test was used, posteriori comparisons for more than two groups were carried out taking into account the Bonferroni correction [9]. In the analysis, the criteria for a bilateral critical area were used, the critical level of significance was 0.05. Statistical analysis of the research results was carried out in the statistical package EZR v. 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan, 2020) [10], which

provides a graphical interface to R (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

It is known that cellular components, including nucleic acids of xenografts, are strong antigens that trigger immunological reactions in the recipient provoking graft rejection. A decellularization protocol is considered to be effective in removing all cell components and nucleic residues [11]. To assess the decellularization, examination of samples stained with hemotoxylin-eosin and by the DAPI method was performed. Staining with hematoxylin and eosin revealed the presence of visible cell nuclei colored in violet in native pericardium of the control group (Fig. 1). At the same time, histological analysis showed that decellularized pericardial tissues retained the ECM components without cells or nuclei.

Additionally, the absence of relevant DAPI staining confirmed that most cellular nuclear material was removed after emulsification with SDS detergent ($P < 0.05$, 95% CI 84.3% – 100%). The aforementioned could be clearly visualized by microscopic examination of DAPI samples (Fig. 2). We observed the absence of nuclear elements in expiremental group.

Qualitative microstructure analysis of SEM images also confirmed no obvious difference in the structure and distribution between the collagen and elastin fibers of the decellularized extracellular matrix and the native bovine pericardium (Fig. 3).

In the samples of the experimental group, the DNA concentration was estimated at the level of 3 ng/mg on the 35th day of decellularization (Table 1). Thus, the degree of matrix purification of decellularized bovine pericardium from nucleic acid residues was 99.8% compared to native samples. A statistically significant difference was found between the DNA concentrations of the experimental and control groups ($P < 0.05$).

The study of biomechanical properties showed that the decellularized matrix not only did not change its natural properties, but also gained greater strength after the process of purification and cross-linking. This is evidenced by an increase almost 2 times of the maximal tensile strength value in the samples of the experimental group by longitudinal tissue testing (Table I).

The cytotoxicity study of the decellularized extracellular matrix showed that after two months of cultivation the culture of human fibroblast cells formed monolayer on the surface of the BP sample. Some groups of fibroblasts penetrated to a depth of 350 μm of low density fiber bundles (Fig. 4).

The ultimate goal of implantation is bioimplant integration into the host tissue with its subsequent regeneration [12]. Fig. 5 presents a histological examination of the explanted pericardium in control rats implanted with untreated/native BP. As expected, the pericardium is completely degraded and eliminated remaining only connective and muscle tissues. The tissue is infiltrated with leukocytes indicating inflammatory processes in the area.

At the same time, the histology data indicate successful biointegration of the implant in the rats of the experimental group in comparison with the control (Fig. 5). In the tissues, implant replacement with growing immature connective tissue was noted. In the area of implant, the increased vascularization of connective tissue is also observed, capillaries are formed, which are filled with erythrocytes. There was a lower level of macrophages and monocytes filtration in decellularized tissues of the pericardium compared to non decellularized tissues of the control group ($P< 0.05$).

DISCUSSION

The result of this study was obtaining a xenograft scaffold with biomechanical and biological characteristics similar to the native BP. SDS was used in the decellularization process. It is well known that SDS is an ionic detergent, capable of binding to collagen fibers, destroying them and promoting swelling of the tissue, caused by a potential break in hydrogen bonds of the collagen fibers [11, 13]. SDS has been reported to interact strongly with the ECM proteins, making its complete removal challenging [14]. Ning Lia et al. also noted that residual SDS in decellularized tissues could result in an insufficient repopulation with the host cells after implantation, causing a lower durability of the implant and inhibiting growth. This was clearly correlated in rat implantation studies, where peri-implant necrosis was found around the 1% SDS-treated implants [15].

Nevertheless, the literature confirms the effective use of this detergent, aiming to solubilize the cytoplasmic and nuclear elements while maintaining structure-function properties and recellularization potential of the matrix. This study confirms the hypothesis and demonstrates the effective use of low-concentration 0.1% SDS detergent and EDC/NHS cross-linking for decellularization of the BP. Tran Ha Le Bao et al. also showed that 0.1% SDS solution comparing to 0.3% or 0.5% SDS is more appropriate for porcine pericardial decellularization due to better preservation of the scaffold biological properties [16].

The microstructure analysis of hematoxylin-eosin and SEM images also showed the absence of any changes in collagen structure. Based on these facts, we confirmed hypothesis that the application of SDS at low concentration (0.1%) is enough for the effective decellularization. Surprising fact was observed that the decellularized BP had higher biomechanical characteristics comparing the native pericardium. Some scientists reported that collagen fibrils are more densely packed after crosslinking with EDC/NHS and have a more uniform structure comparing to noncrosslinked ones [17]. Besides, the EDC-crosslinked scaffolds possessed excellent mechanical property and biocompatibility.

Earlier studies have reported the cytotoxic nature of glutaraldehyde-treated tissue without indicating which fraction is cytotoxic [4]. In this case we used glutaraldehyde-free cross-linking. We noticed not only the absence of scaffold toxic effects on fibroblasts cells, but an increase

of cell concentration on decellularized matrix. At the same time, histological data indicate successful integration of the implant in rats.

CONCLUSIONS

BP was successfully decellularized with additional application of cross-linking agent NHC/EDC maintaining structure-function properties and recellularization potential. Overall, BP tissue decellularized with 0.1 % SDS and NHS/EDC cross-linking is promising in the manufacturing of tissue-engineered materials for cardiac surgery.

REFERENCES

1. Pawan K.C., Hong Y., Zhang G. Cardiac tissue-derived extracellular matrix scaffolds for myocardial repair: advantages and challenges. Regen Biomater. 2019; 6(4): 185-199. doi: 10.1093/rb/rbz017.
2. Sarig U., Au-Yeung G.C.T., Wang Y. et al. Thick acellular heart extracellular matrix with inherent vasculature: a potential platform for myocardial tissue regeneration. Tissue Eng Part A. 2012;18:2125–37.
3. Gratzer P.F., Harrison R.D., Woods T. Matrix alteration and not residual sodium dodecyl sulfate cytotoxicity affects the cellular repopulation of a Decellularized matrix. Tissue Eng. 2006;12:2975.
4. Gendler E., Gendler S., Nimmi M.E. Toxic reactions evoked by glutaraldehyde fixed pericardium and cardiac valve tissue bioprostheses. J Biomed Mater Res. 1984;18:727–36.
5. Choe J.A., Jana S., Tefft B.J. et al. Biomaterial characterization of off–the–shelf decellularized porcine pericardial tissue for use in prosthetic valvular applications.J Tissue Eng Regen Med. 2018;12(7): 1608–1620. doi: 10.1002/term.2686.
6. Park S., Kim S.H., Lim H.G. et al. The Anti-calcification Effect of Dithiobispropionimidate, Carbodiimide and Ultraviolet Irradiation Cross-linking Compared to Glutaraldehyde in Rabbit Implantation Models. Korean J Thorac Cardiovasc Surg. 2013;46(1):1-13. doi: 10.5090/kjtcs.2013.46.1.1.
7. Vunjak-Novakovic G., Freshney R.I. Culture of Cells for Tissue Engineering. 2006. 536 p.
8. Korzhevsky D. E. Application of hematoxylin in histological technique. Morphology. 2007; 132(6):77–82. (in Russian).
9. Guryanov V.G., Lyakh Yu. E., Pariy V.D. et al. Tcekhmister Handbook of Biostatistics. Analysis of the results of medical research in the package EZR (R – statistics). Textbook. – K.: Vistka; 2018, 208 p.
10. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452–458.
11. Gilbert W.T., Sellaro L.T., Badylak F.S. Decellularization of tissues and organs. Biomaterials. 2006;27:3675–3683. doi:10.1016/j.biomaterials.2006.02.014.
12. Rakhmatia Y.D., Ayukawa Y., Furuhashi A., Koyano K. Current barrier membranes:titanium mesh and other membranes for guided bone regeneration in dental applications. J Prosthodont Res. 2013;57:3–14. doi:pmid:23347794. (in Russian).
13. Oswal D., Korossis S., Mirsadraee S. et al. Biomechanical characterization of decellularized and cross-liked bovine pericardium. J. Heart Valve Dis. 2007;16:165–174.
14. Andre´e B., Bela K., Horvath T. et al. Successful re-endothelialization of a perfusable biological vascularized matrix (BioVaM) for the generation of 3D artificial cardiac tissue. Basic Res Cardiol. 2014;109:441.
15. Lia N., Lia Y., Gong D. et al. Efficient decellularization for bovine pericardium with extracellular matrix preservation and good biocompatibility. Interactive CardioVascular and Thoracic Surgery 2018;26:68–776. doi:10.1093/icvts/ivx416.
16. Tran H.L.B., Dihn T.H., Nguyen T.N. et. al. Preparation and characterization of acellular porcine pericardium for cardiovascular surgery. Turk J Biol. 2016;40:1243-1250. doi:10.3906/biy-1510-44.
17. Nong L.M., Zhou D., Zheng D. et al. The effect of different cross-linking conditions of EDC/NHS on type II collagen scaffolds: an in vitro evaluation. Cell and Tissue Banking. 2019;20:557-568.

ORCID and contributionship:

Nataliia V. Shchotkina: 0000-0002-8564-5881 [B,D]
Anatoliy A. Sokol: 0000-0002-4232-4025 [C,E]
Oleksandr Yu. Galkin: 0000-0002-5309-6099 [A, F]
Glib I. Yemets: 0000-0002-6139-6235 [E]
Liudmyla V. Dolinchuk: 0000-0003-3006-0832 [E]
Nadiia M. Rudenko: 0000-0002-1681-598X [F]
Iliia M. Yemets: 0000-0002-1893-0164 [A,F]

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CORRESPONDING AUTHOR

Nataliia V. Shchotkina
Center for Pediatric Cardiology and Cardio Surgery
24 Yuri Ilyenko st., 04050 Kyiv, Ukraine
tel: + 380502599672
e-mail: carditissue@gmail.com

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ORIGINAL ARTICLE

MORPHOLOGICAL FEATURES OF THE UTERUS IN WOMEN AT DIFFERENT TIME INTERVALS OF THE POSTMORTEM PERIOD AS DIAGNOSTIC CRITERIA FOR ESTABLISHING THE POSTMORTEM INTERVAL

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Vasil O. Olkhovsky[1], Edgar K. Grygorian[1], Mykhailo S. Myroshnychenko[1], Sergii V. Kozlov[2], Kostiantyn M. Suloiev[2], Anton O. Polianskyi[3], Petro A. Kaplunovskyi[1], Yuliia Ya. Fedulenkova[1], Iryna V. Borzenkova[4]

[1]KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

[2]STATE INSTITUTION «DNIPROPETROVSK MEDICAL ACADEMY OF THE MINISTRY OF HEALTH OF UKRAINE», DNIPRO, UKRAINE

[3]NATIONAL SCIENTIFIC CENTER «HON. PROF. M. S. BOKARIUS FORENSIC SCIENCE INSTITUTE», KHARKIV, UKRAINE

[4]PUBLIC NONPROFIT ORGANIZATION OF THE KHARKIV DISTRICT COUNCIL «REGIONAL CLINICAL HOSPITAL», KHARKIV, UKRAINE

ABSTRACT

The aim is to identify the morphological features of the uterus layers in women at different time intervals of the postmortem period as diagnostic criteria for establishing the postmortem interval.

Materials and methods: In the study we used surgical and autopsy material – uterine tissue fragments. All materials were divided into two groups. The 1st group (G 1) included surgical material from women (n=6) who underwent removal of the uterus, or uterus with the appendages due to leiomyoma, uterine prolapse. The 2nd group (G 2) included autopsy material from 42 women with known causes of death and postmortem period (from 24 to 48 hours – 6 cases, from 49 to 72 hours – 7 cases, from 73 to 96 hours – 8 cases, from 97 to 120 hours – 6 cases, from 121 to 144 hours – 8 cases, more than 144 hours – 7 cases). Histological and immunohistochemical study methods were used.

Results: A comprehensive morphological study of the women uterus revealed a time-dependent increase of postmortem changes in this organ linked with the increase of postmortem period. In cases of postmortem period duration up to 144 hours, the structural elements of the uterine layers were identified. In cases where the duration of the postmortem period was more than 145 hours, microscopically the uterus was represented by eosinophilic fibrous or dusty masses, the histogenesis of which could not be determined. The processes of autolysis occurred more intensely and faster in the mucous membrane of the uterus, in comparison with the muscular and serous membranes, and in the vessels – in their inner membrane, in comparison with the middle and outer membranes. Autolytic changes in the muscular membrane of the uterus and vascular walls occurred more intensely in muscle fibers compared to connective tissue fibers.

Conclusions: The histological and immunohistochemical features of the women uterus at different postmortem periods have a certain forensic medical significance and can be used for establishing the postmortem interval.

KEY WORDS: morphological features, uterus, postmortem period, diagnostic criteria

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INTRODUCTION

The issues of determining the postmortem interval in forensic science and practice were always relevant and will probably remain so for a long time [1]. The prescription of death, as is known, is determined by a forensic expert when examining a corpse at the place of its detection and its subsequent autopsy [2].

Today, there is a large arsenal of diagnostic methods that allow forensic experts to establish approximate time intervals for the prescription of death. Some methods are quite complex for practical use, while others are only of scientific and theoretical significance, since their implementation requires complex and expensive equipment, which is used in special fields of science and technology [3, 4]. Thus, an important issue of modern forensic science and practice is the search and development of new and effective diagnostic criteria that would allow experts to determine the postmortem interval more accurately and to give a reasoned opinion meeting the needs of law enforcement agencies.

An important role in the development of diagnostic criteria that allow experts to establish the postmortem interval in various terms of the postmortem period is assigned to morphological methods. In the available domestic and foreign literature, we did not identify the complex morphological studies of the uterus in order to establish the valid and reliable diagnostic criteria for determining the postmortem interval. That fact actualizes the current study.

THE AIM

The aim is to identify the morphological features of the uterus layers in women at different time intervals of the postmortem period as diagnostic criteria for establishing the postmortem interval.

MATERIALS AND METHODS

In the study we used surgical and autopsy material – uterine tissue fragments. All materials were divided into two groups. The 1st group (G 1) included surgical material from women (n=6) who underwent removal of the uterus, or uterus with the appendages due to leiomyoma, uterine prolapse. The 2nd group (G 2) included autopsy material from 42 women with known causes of death and postmortem period (from 24 to 48 hours – 6 cases, from 49 to 72 hours – 7 cases, from 73 to 96 hours – 8 cases, from 97 to 120 hours – 6 cases, from 121 to 144 hours – 8 cases, more than 144 hours – 7 cases). The average age of women in G 1 and G 2 did not have significant differences ($p>0.05$) and amounted to 68.7±2.5 years and 69.4±2.7 years, respectively, which, according to the age classification of the World Health Organization, corresponds to old age. The criteria for excluding cases from G 1 were the presence of information in the medical history about the woman's intake of alcohol or drugs, from G 2 – the presence of information about the woman's intake of alcohol or drugs, violent death or death with an unknown cause and diagnosis.

In G 1 and G 2 the fragments of uterine tissue were cut out through the entire thickness of the organ in the areas without macroscopically detected pathological processes. The resulting material was fixed in a 10 % solution of neutral formalin (ph 7.4) for 24-48 hours, carried out according to the generally accepted technique and embedded in paraffin. From paraffin blocks serial sections with a thickness of 4-5 μm were made. The slides stained with hematoxylin and eosin were examined using an Olympus BX-41 microscope (Japan).

Immunohistochemical study was performed according to standardized protocols using a murine monoclonal antibody (MCA) to smooth muscle actin (HHF35 clone, Diagnostic BioSystems Inc., USA). Visualization was performed using a PolyVue Plus™ HRP/DAB Detection System (Diagnostic BioSystems Inc., USA).

RESULTS

In G 1, an observation microscopy in the uterus revealed three characteristic layers (mucosa, or endometrium; muscular, or myometrium; serous, or perimetrium), the boundaries between which were clear (fig. 1a, 1b). Among these layers, myometrium was characterized by the greatest thickness. In all uterus layers, stained with hematoxylin and eosin, there was a uniform staining of cell nuclei in a blue and violet color and the cells cytoplasm, connective tissue fibers in a pink-red color.

The endometrium was lined with a single-layer prismatic epithelium, which was located on the loose unformed connective tissue. Vessels, nerve fibers and numerous uterine glands, extending through the entire thickness of the endometrium, passed between the connective tissue fibers. The uterine glands in some fields of view looked like narrow straight tubes or cystic-enlarged cavities, and in some fields of view they lengthened and twisted in a corkscrew-like manner. Also, between the connective tissue fibers, a diffuse polymorphic cellular infiltration was revealed, which mainly consisted of the cells of fibroblast series and immune cells.

The myometrium was characterized by the presence of parenchyma and stroma. The parenchymal component was represented by a set of smooth muscle cells, which united into bundles and formed three layers, the boundaries between which were indistinct. The direction of smooth muscle cells in the layers was different: longitudinal – in the outer and inner layers, circular – in the middle layer. In most fields of view, the bundles of muscle fibers were tightly attached to each other. Myocytes were characterized by the presence of a rod-shaped nucleus located in the central part of the cell. The stroma was represented by thin layers of connective tissue fibers, between which vessels and nerve fibers were located. Also diffuse, moderately pronounced polymorphic cellular infiltration was determined, represented mainly by the cells of fibroblast series and immune cells.

The serous layer was adjacent to the myometrium and was represented by densely located connective tissue fibers. Between them, there were nerve fibers, vessels and polymorphic-cellular infiltration, which was represented mainly by the cells of fibroblast series and immune cells. From the surface, the serous layer was covered by mesothelium.

In G 1, an immunohistochemical reaction with MCA to smooth muscle actin showed the uniform and clear cytoplasmic dark brown staining of myofibroblasts located in all layers of the uterus, as well as of smooth muscle cells of the vessels and myometrium.

In G 2, in cases where the postmortem period lasted from 24 to 48 hours, an observation microscopy revealed clear boundaries between the uterus layers. The endometrium was characterized by uneven thickness (fig. 2a). It lacked the characteristic single-layer prismatic epithelium. In the lamina propria there were moderate edematous changes of the cellular elements and connective tissue fibers, which uneven stained with hematoxylin and eosin. Light slit-like spaces were identified between the connective tissue fibers. Stromal cellularity did not change compared with G 1. In most of the uterine glands, desquamation of epithelial cells was detected. Desquamated cells were located in groups in the glands lumens, had a well-defined structure or were enlarged in size with pyknotic nuclei. In the lumens of some glands, only the epithelial cells nuclei were determined, which indicated about the violation of the integrity of these cells.

In myometrium, it was revealed uneven staining with hematoxylin and eosin of the cells nuclei and cytoplasm, as well as connective tissue fibers (fig. 2b). In this membrane, moderate edema of muscle and connective tissue fibers with the presence of light slit-like spaces between them was identified. Muscle fibers in some field of views were also characterized by the presence of few foci of wave-like deformations, thinning and partial ruptures across the fiber axis. In some fields of view, the nuclei of muscle cells were increased in size with light purple color, and in other places they were small with dark purple color. In some myocytes,

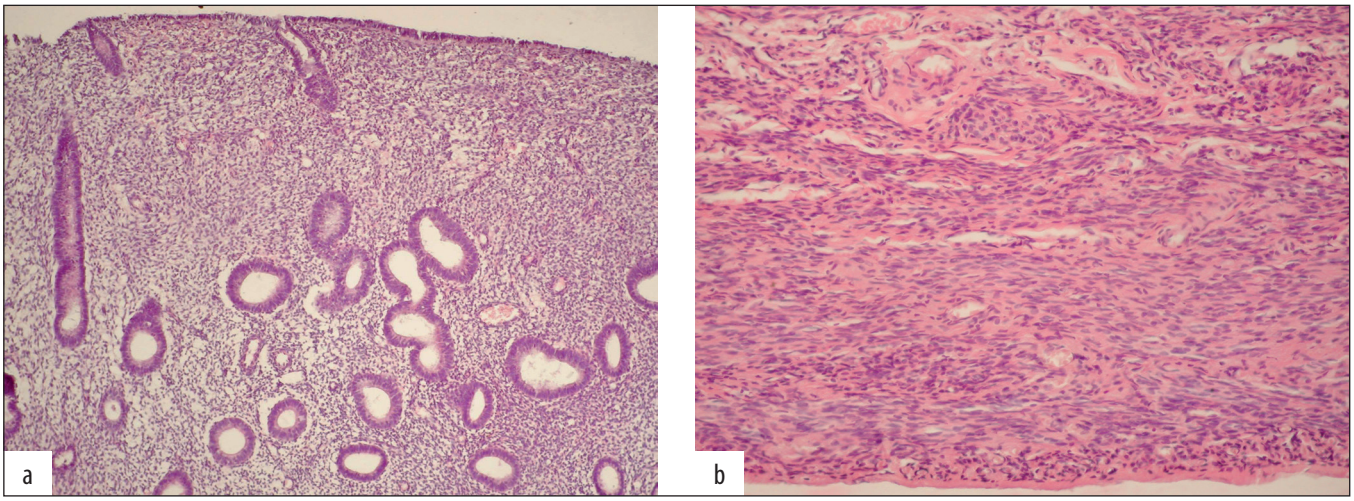

Fig. 1. G 1. Microscopic structure of the mucous, muscular and serous layers of the uterus. Hematoxylin and eosin staining, a) ×100, b) ×200.

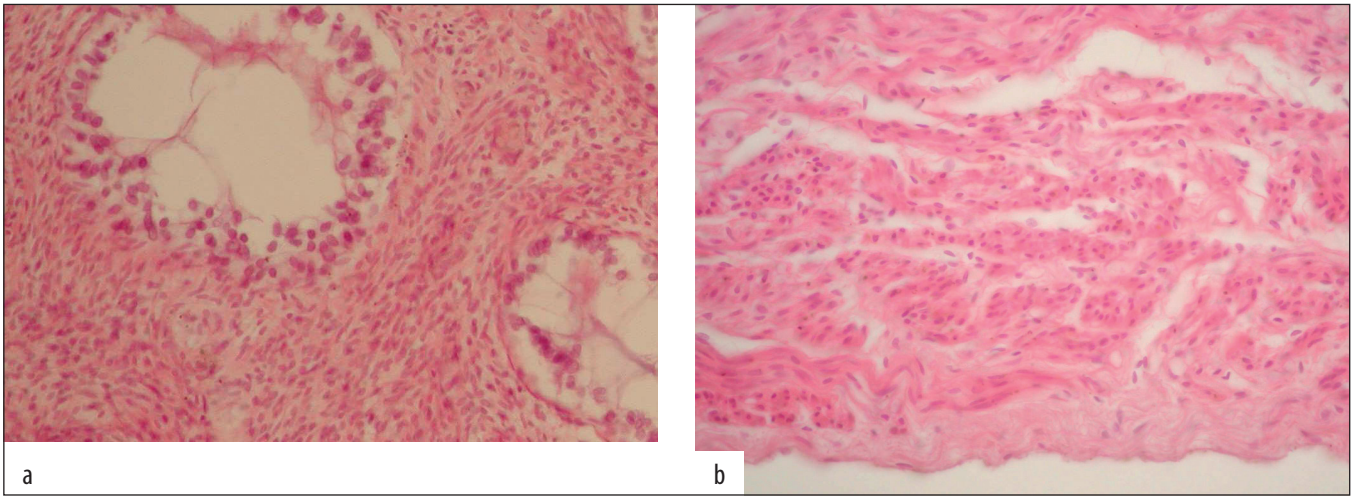

Fig. 2. G 2. The duration of the postmortem period was 39 hours. Microscopic structure of the mucous, muscular and serous layers of the uterus. Hematoxylin and eosin staining, a) ×400, b) ×400.

nuclei were not detected. Between the connective tissue fibers, as in G 1, the cells of fibroblast series and immune cells with moderate edematous changes were visualized.

In serous layer, the mesothelium was practically undetectable (fig. 2b). Moderate edematous changes of cellular elements and connective tissue fibers led to their uneven staining with hematoxylin and eosin. In the light slit-like spaces between the connective tissue fibers, the cells of fibroblast series and immune cells were determined, the number of which was similar to G 1.

In most of the vessels located in all layers of the uterus, there were pronounced endothelial cells desquamation, moderate edematous changes of connective tissue and muscle fibers, and the absence of nuclei in some of the myocytes. In the lumens of most vessels, desquamated endothelial cells of an elongated-curved shape and unchanged erythrocytes were found.

The expression of smooth muscle actin was determined in myofibroblasts, smooth muscle cells of the vessels and myometrium. However, in the described cases, in comparison with G 1, a decrease of the smooth muscle actin expression was revealed in some fields of view, as a result of which these areas had a light brown color.

In cases where the postmortem period ranged from 49 to 72 hours, in comparison with the earlier postmortem period, we found more pronounced edematous changes and uneven staining of cellular elements and connective tissue fibers with hematoxylin and eosin in all uterine layers.

In mucous membrane we determined the deformed glands with ruptures. There were an increase the desquamative changes of epithelial cells, which were located in the glands lumen and characterized by the absence of nuclei or the presence of dark-violet nuclei of reduced in size, turbid cytoplasm and indistinct contours. It was revealed the increased number of glands with the presence of a fragment of epithelial cells in their lumens, reduced in size nuclei with the clear areas in their central part (fig. 3). Cellularity of stroma was decreased. The connective tissue fibers were with a loss of the characteristic clarity of the contours, their fragmentation. Also there were increased the spaces between the connective tissue fibers.

In myometrium it was revealed an increase the number

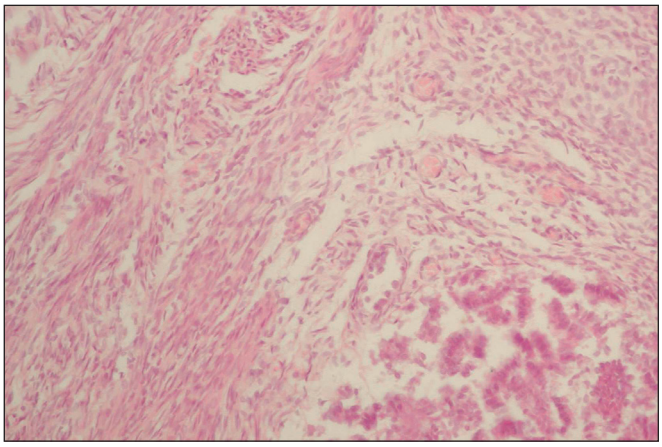

Fig. 3. G 2. The duration of the postmortem period was 68 hours. Microscopic structure of the mucous and muscular layers of the uterus. Hematoxylin and eosin staining, ×400.

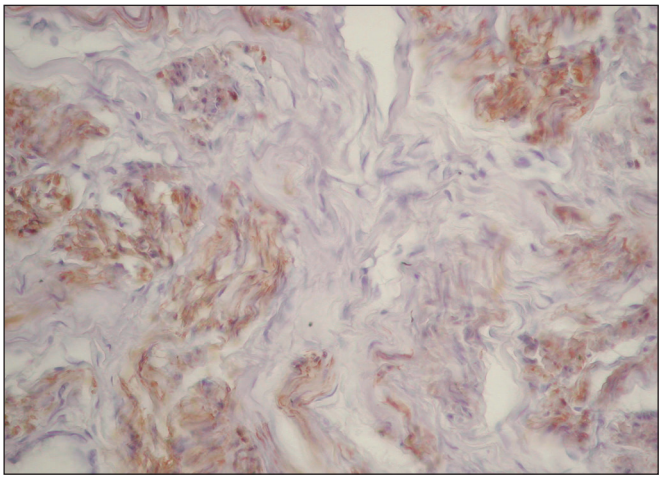

Fig. 4. G 2. The duration of the postmortem period was 85 hours. Immunohistochemical reaction with MCA to smooth muscle actin, ×400.

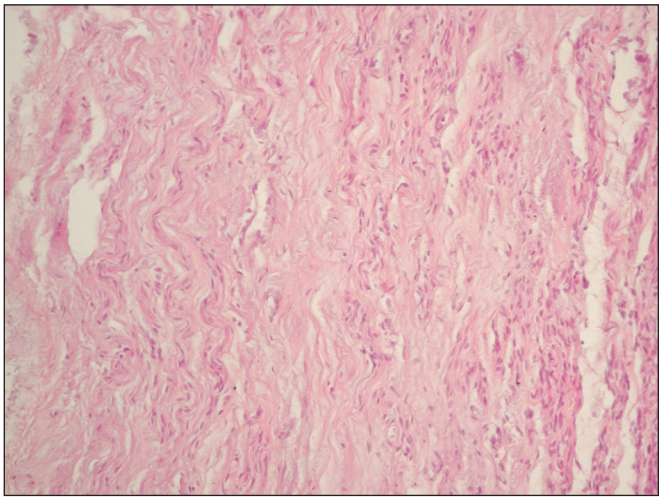

Fig. 5. G 2. The duration of the postmortem period was 110 hours. Microscopic structure of the myometrium. Hematoxylin and eosin staining, ×200.

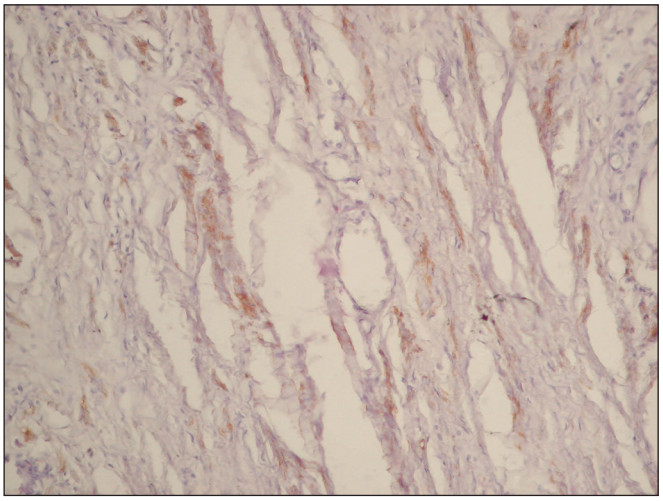

Fig. 6. G 2. The duration of the postmortem period was 136 hours. Immunohistochemical reaction with MCA to smooth muscle actin, ×200.

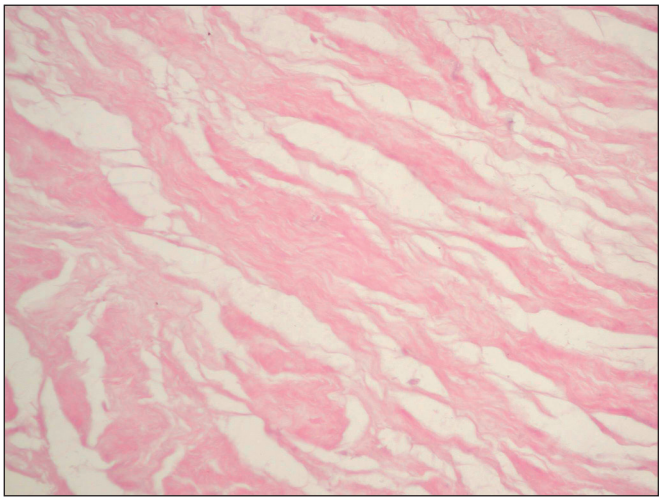

Fig. 7. G 2. The duration of the postmortem period was 155 hours. Hematoxylin and eosin staining, ×200.

of the fields of view with the wave-like deformations of the muscular fibers, swelling and turbidity of the their cytoplasm, lack of characteristic clarity of their contours, their fragmentation and partial ruptures. It was determined the muscle fibers with a complete rupture and disintegration into separate segments. Myocyte nuclei decreased in size and were light purple. We found an increase the number of non-nuclear myocytes. The myocytes nuclei appeared in the intercellular spaces. It was revealed an increase the spaces between the muscle fibers (fig. 3).

In stroma of the myometrium, as well as in serous layer, it was noted an increase the connective tissue fibers swelling. Connective tissue fibers disintegrated into the separate fragments. The spaces between the connective tissue fibers continued to increase. Cellularity decreased markedly.

In the vessels that were located in all uterine layers, the endothelium was not visualized. There was an increase the muscle and connective tissue fibers swelling. It was appeared the rupture and loss of the muscle and connective tissue fibers contour visibility. We found an increase the number of non-nuclear myocytes. An increase the number of desquamated endothelial cells and the appearance of hemolyzed erythrocytes were noted in the vessels lumens.

An immunohistochemical study revealed a decreased expression of smooth muscle actin by myofibroblasts,

smooth muscle cells of the myometrium and vessels, which was manifested by an increase the number of light brown areas. In few fields of view the expression of that MCA was virtually undetected.

In cases where the duration of the postmortem period ranged from 73 to 96 hours, microscopically in all uterine layers there were fields of view with structures unevenly stained with hematoxylin and eosin. Besides, the number of such fields of view in these cases was larger compared to cases where the duration of the postmortem period ranged from 49 to 72 hours. Also in these cases in myometrium and walls of the vessels during hematoxylin and eosin staining there were some fields of view with light pink color and the presence of shadow nuclei, in which it was impossible to differentiate the muscle and connective tissues.

The mucous membrane of the uterus in these cases was not detected in some fields of view. In the fields of view, where that membrane was detected, in comparison with previous cases, there were an increase the number of glands with ruptures and desquamative changes in epithelium of the glands, as a result of which, in their spaces, the small formless elements or strata without a pronounced structure were observed. Some glands took the shape of round-oval voids of various sizes.

In stroma of the endometrium edematous changes of the cellular elements and connective tissue fibers increased, which led to the swelling of the latte. The cellularity was sharply decreased, which in some fields of view was not determined. There was an increase the number of fields of view with thinning, ruptures and fragmentation of connective tissue fibers into separate particles, the boundaries of which in most cases were indistinguishable. The spaces increased between the connective tissue fibers.

In parenchyma of the myometrium, edematous changes of muscle fibers increased, which led to their swelling. Wave-like deformation and complete ruptures of muscle fibers, that did not have clear contours, were growing. It was an increase the number of non-nuclear myocytes and myocytes with the presence of shadow nuclei. These were also an increase the number of the fields of view with the presence in the intercellular space of reduced nuclei of light violet color, or fragments of nuclei. The intermuscular spaces became larger and in some fields of view took the form of cavities.

In connective tissue fibers of muscle and serous layers, there were an increase edematous changes and their swelling. These fibers continued to lose the characteristic clear boundaries and to grow their disintegration into the separate fragments. Between the connective tissue fibers, the spaces increased, which in some fields of view looked like cavities. The cellularity sharply decreased, which was not detected in some fields of view.

In vessels located in all layers of the uterus, there were an increase edematous changes and swelling, ruptures and loss of clarity contours of muscle and connective tissue fibers. We found an increase the number of non-nuclear myocytes and myocytes with the presence of shadow nuclei. In lumens of the vessels, erythrocytes-shadows were identified.

The expression of smooth muscle actin by myofibroblasts, smooth muscle cells of the vessels and myometrium was sharply reduced. In a significant number of fields of view, the number of areas with a positive reaction sharply decreased. The latter were light brown color (fig. 4).

In cases where the duration of the postmortem period ranged from 97 to 120 hours, microscopically the mucous membrane was not determined in the uterus. The boundaries between the muscular and serous layers were indistinct. In comparison with the previous cases, in these cases there was an increase the unevenness of staining of structural elements with hematoxylin and eosin.

In myometrium, in a significant number of fields of view there was an increase the number of homogeneous areas of light pink color. In those areas it was impossible to differentiate the muscle and connective tissues (fig. 5). In single fields of view, where muscle and connective tissue were visualized, in muscle fibers there were an increase edema and swelling, wave-like deformation, fragmentation, loss of contours clarity. The number of myocytes without nuclei and myocytes with shadow nuclei increased. The width of the intermuscular spaces increased, and they often had a cavity-like shape.

In the connective tissue fibers of myometrium and perimetrium, swelling, fragmentation, loss of contours clarity and ruptures were increased. In these layers only in some fields of view the cells of fibroblast series and immune cells were detected.

Vessels located in all layers of the uterus in some fields of view had a totally homogeneous wall of light pink color, in which the layers were not contoured. In some of the vessels, predominantly of large caliber, the contouring of the layers was noted, in there, the muscle and connective tissue fibers were characterized by swelling, indistinct contours and ruptures. The number of non-nuclear myocytes, myocytes with the presence of shadow nuclei, myocytes with reduced in size nuclei of light purple color increased. Lumens of the vessels were without any content or, in some places, erythrocytes-shadows were determined.

In single fields of view, a few smooth muscle cells of the muscle layer expressed smooth muscle actin. These areas looked light brown.

In cases where the duration of the postmortem period ranged from 121 to 144 hours, when stained with hematoxylin and eosin, in all fields of view the eosinophilic (light pink) nuclear-free fibrous structures or their fragments were revealed in the muscular layer that was located closer to the serous membrane. In these areas, it was not possible to differentiate the muscle and connective tissue components, and single basophilic structures that were localized between the fibers fragments which resembled the structure of immune cells. There were wide spaces or cavities between the above-mentioned fibrous structures.

In the deeper parts of the myometrium, located in the direction or closer to the mucous membrane, against the background of eosinophilic fragments of fibrous structures, the histogenesis of which could not be determined, the single areas with the presence of small fragments of muscle

and connective tissue fibers were revealed. The latter did not have clear boundaries, looked swollen and fragmented. In a part of myocytes, small nuclei of light violet color without clear boundaries or shadow nuclei were revealed. The spaces between the fibers were wide. Between the fibers, in some places there were single immune cells.

When stained with hematoxylin and eosin, the serous membrane was represented in a significant number of the fields of view by eosinophilic (pale pink) fragments of fibrous structures that did not have clear contours and were located chaotically. Also, in this layer, single basophilic (light purple) structures were identified the structure of which most likely corresponded to immune cells.

The vessels located in all layers had a rounded-oval shape and were characterized by the presence of light pink fibers, among which it was impossible to differentiate muscle and connective tissues. Fibrous structures often looked fragmented and swollen, with breaks. There was no content in the lumens of the vessels.

When staging an immunohistochemical reaction with smooth muscle actin, its expression by single smooth muscle cells of the muscular layer was revealed. These myocytes were uniformly light brown or contained light brown granules. In a significant number of the fields of view, the expression of this MCA was not detected (fig. 6).

In cases where the duration of the postmortem period was more than 145 hours, when stained with hematoxylin and eosin, it was impossible to differentiate the muscular and serous layers. The basophilic structures were not determined (fig. 7). Only fibrous structures or dusty masses of light pink were noted. The fibrous structures, totally, looked sharply swollen, with indistinct contours and with the areas of breaks. Small vessels were not visualized, while large vessels were identified due to their rounded or rounded-oval shapes.

The expression of smooth muscle actin was not determined.

DISCUSSION

Autolysis processes that occur after death are a special feature of biological objects to hydrolytically decompose their own molecular and cellular structures. The autolytic process, as is known, is usually divided into several stages depending on the time of death (the stage of initial changes, pronounced changes and the stage of late autolytic changes), each of which is characterized by certain biochemical, biophysical, morphological and immunohistochemical changes [5].

During this study, the features of morphological changes in the mucous, muscular and serous layers of the uterus of elderly women at different periods of the postmortem period were defined for the first time. The authors found that the processes of autolysis in the uterus occur more intensively and faster in the mucous membrane compared to the muscular and serous membranes, which was confirmed by the absence of this membrane during microscopic examination, in cases where the duration of the postmortem period was 97 hours or more.

The study of the morpho-functional features of the endometrium in forensic science and practice allowed examiners to obtain additional information about the age, obstetric and gynecological data and some phenotypic features of the identified personality [6].

In our study, it was noted that in the muscular layer of the uterus, as well as in the walls of the vessels located in all its layers, autolytic changes occurred more intensively in muscle fibers in comparison with connective tissue fibers. This fact, from our point of view, is due to the structural features of the connective tissue (its high density, poor hydrophilicity).

Delayed autolytic changes in connective tissue were noted by a number of scientists when studying the morphological features of the Achilles tendon at various times of the postmortem period [7]. Resistance to postmortem changes in connective tissue and foci of sclerosis was also noted by scientists during a morphological study of the kidneys [8].

Scientists studying postmortem changes in the skeletal muscles of rats during postmortem autolysis showed rapid changes in the properties of actin and myosin, which are known to be proteins constituting the structural basis of myofibrils [9].

Immunohistochemical studies are a useful tool for more accurate, in comparison with other morphological methods, determination of the age of various types of wounds, postmortem interval, etc. [10]. The authors' immunohistochemical study with MCA to smooth muscle actin made it possible to identify myofibroblasts located in all layers of the uterus, as well as to differentiate muscle and connective tissue fibers in the walls of blood vessels and the myometrium.

CONCLUSIONS

1. A comprehensive morphological study of the women uterus revealed a time-dependent increase of postmortem changes in this organ linked with the increase of postmortem period. In cases of postmortem period duration up to 144 hours, the structural elements of the uterine layers were identified. In cases where the duration of the postmortem period was more than 145 hours, microscopically the uterus was represented by eosinophilic fibrous or dusty masses, the histogenesis of which could not be determined. The processes of autolysis occurred more intensely and faster in the mucous membrane of the uterus, in comparison with the muscular and serous membranes, and in the vessels – in their inner membrane, in comparison with the middle and outer membranes. Autolytic changes in the muscular membrane of the uterus and vascular walls occurred more intensely in muscle fibers compared to connective tissue fibers.
2. The histological and immunohistochemical features of the uterus of women at different postmortem periods have a certain forensic medical significance and can be used for establishing the postmortem interval.

The prospect for further research is the development of a computer program for analyzing the color properties of raster images, which will allow, when examining slides, to identify qualitative characteristics and, as a result, to carry out the accurate and correct morphological diagnostics of processes or phenomena, as well as perform a statistical analysis of the data obtained.

REFERENCES

1. Mishalov VD, Dunaev OV, Kostenko EYa, Kozlov SV. Perspektyvy doslidzhennja biologichnyh ridyn dlja vyznachennja davnosti smerti. Prospects for studying biological fluids to determine the death prescription. Ukrainian Journal of Medicine, Biology and Sport. 2019;4(4/20):218-223. (Ua).
2. Kildyushov EM. Modelyrovanye posmertnogo processa teploobmena y opredelenye davnosty nastuplenyja smerty. Modeling the postmortem heat transfer process and determining the death prescription. Immanuel Kant Baltic Federal University Vestnik. 2014;7:128-134. (Ru).
3. Bachynskiy VT, Ushenko OG, Vanchuliak OY, Sarkisova YV. Zastosuvannja novyh perspektyvnyh tehnologij dlja vyznachennja davnosti nastannja smerti v praktyci sudovo-medychnogo eksperta. Application of perspective new technologies to determine the time of death in practice of forensic medical experts. Clinical and Experimental Pathology. 2017;16(1/59):157-159. (Ua).
4. Olkhovskiy VO, Golubovic LL, Tagaev NN et al. Vyznachennja davnosti nastannja smerti. Determing the statute of limitations for death. Kharkiv: FOP Brovin OV, 2017. 168 p. (Ua).
5. Byvaltsev VA, Stepanov IA, Semenov AV et al. Vozmozhnosti diagnostiki davnosti nastuplenija smerti po izmenenijam v pojasnichnyh mezhpozvonkovyh diskah (sopostavlenie morfologicheskih, immunogistohimicheskih i tomograficheskih rezultatov). [The possibilities for diagnostics of prescription of death coming based on the changes in the lumbar intervertebral disks (the comparison of the morphological, immunohistochemical and topographical findings).] Forensic Medical Expertise. 2017;4:4-8. (Ru).
6. Nedugov HV, Nedugova VV. Issledovanie funkcionalnoj morfologii jendometrija v celjah identifikacii lichnosti. Investigation of the endometrial morphology for the personal identification. Problems of expertise in medicine. 2003;2:18-20. (Ru).
7. Povstyaniy V. Dynamika mikroskopichnyh zmin Ahillovogo suhozhyku uprodovzh dvoh tyzhniv pislja nastannja smerti. Dynamics of microscopic changes of the Achilles tendon for two weeks after the death. Forensic examination. 2016;2:38-43. (Ua).
8. Kildyushov EM, Ermakova YuV, Tumanov EV, Kuznetsova GS. Diagnostika davnosti nastuplenija smerti v pozdnem posmertnom periode v sudebno-medicinskoj praktike (obzor literatury). Estimation of time since death in the late postmortem period in forensic practice (literature review). Russian Journal of Forensic Medicine. 2018;4(1):34-38. (Ru).
9. Nikitina ZK, Abrosimenkova NN, Rebrov LB. Izmenenija aktina skeletnyh myshc krys v processe posmertnogo autoliza. Alterations in actin of rat skeletal muscles during postmortem autolysis. Voprosy meditsinskoi khimii. 1990;36(2):65-68. (Ru).
10. Fedulova MV, Kupriyanov DD. Dostovernost immunogistohimicheskoj ocenki prizhiznennosti i davnosti povrezhdenij: analiz i perspektivy issledovanij. Reliability of the immunohistochemical estimation of vitality and injury age: analysis and perspectives for study. Forensic Medical Expertise. 2020;63(2):52-57. (Ru).

ORCID and contibutionship:

Vasil O. Olkhovsky – 0000-0003-2843-2692 [A,F]
Edgar K. Grygorian – 0000-0001-5634-015X [A,D]
Mykhailo S. Myroshnychenko – 0000-0002-6920-8374 [D,F]
Sergii V. Kozlov – 0000-0002-7619-4302 [B,E]
Kostiantyn M. Suloiev – 0000-0002-6841-3632 [C,E]
Anton O. Polianskyi – 0000-0003-3005-8206 [E]
Petro A. Kaplunovskyi – 0000-0002-8060-7982 [B]
Yuliia Ya. Fedulenkova – 0000-0001-8599-9500 [B,F]
Iryna V. Borzenkova – 0000-0002-0701-5286 [C,D]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mykhailo S. Myroshnychenko
Pathological Anatomy Department,
Kharkiv National Medical University,
Str. Svetlaya 27A, apt. 70, 61129, Kharkiv, Ukraine
tel: +380501699763, +380961033038
e-mail: msmyroshnychenko@ukr.net

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ORIGINAL ARTICLE

SPECIFIC FEATURES OF UROGENITAL MICROBIOCENOSIS IN WOMEN WITH UROGENITAL MIXED INFECTIONS

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Nataliya Yu. Bysaha[1], Oxana Ol. Korchynska[1], Stefania Andrascikova[2], Silvia Zultakova[2], Alena Schlosserova[2]
[1]DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, MEDICAL FACULTY, UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]PRESHOV UNIVERSITY, PRESHOV, SLOVAKIA

ABSTRACT

The aim: Studying the features of urogenital microbiocenosis in women with urogenital mixed infections.

Materials and methods: In an open comparative randomized study, we surveyed 90 women of childbearing age who sought outpatient treatment at a women's health clinic in Uzhhorod.

Results: During studying the urogenital microflora of the above groups, the following pattern was observed: patients of the main group had a violation of the resident to facultative flora ratio (dysbiosis) on the background of borderline changes in the vaginal mucosa, mainly due to hormonal imbalance manifested by pathological vaginal discharge; instead, in the control group patients, the urogenital flora was saturated with a significant number of lactobacilli (90%).

Conclusions: The women urogenital tract damage by several pathogens simultaneously has been found.

KEY WORDS: urogenital infections, microbiota, inflammatory diseases of pelvic organs

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INTRODUCTION

The term "urogenital infections" includes diseases characterized by damage to the mucous membranes of the urinary tract and genital tract. The course of these diseases is often complicated by involvement in the inflammatory process of the upper genital and urinary tracts, as well as the rectum. Urogenital infections pose a real threat to reproductive health, as they can cause infertility, complicated pregnancy and miscarriages. According to modern ideas and the results of numerous studies, chronic inflammatory process of the genitals many times increases the risk of HIV infection [1–3].

Risk factors that contribute to the development of this pathology should be early sexual life in adolescents, frequent change of sexual partners, neglect of barrier contraception, alcohol, smoking, drug addiction, vitamin deficiency, malnutrition, stress, decreased immunity [4, 5].

All of the above suggests that chronic inflammatory diseases of the female genital organs lead to severe changes in a woman's reproductive system – infertility, menstrual dysfunction, polycystic ovaries, uterine fibroids, endometrial hyperplastic processes, formation of joints in the area of pelvic organs. The anatomical location of the external genitalia and vagina – their close proximity to the anus and urinary tract – provides a high frequency of microorganism exchange between the intestine and urogenital tract, which also contributes to the spread of infection.

THE AIM

Studying the features of urogenital microbiocenosis in women with urogenital mixed infections.

MATERIALS AND METHODS

In an open comparative randomized study, we surveyed 90 women of childbearing age who sought outpatient treatment at a women's health clinic in Uzhhorod.

The patients were divided into two representative clinical groups: the main group consisted of 60 patients with complaints of inflammatory urogenital tract lesions; the control group included 30 patients without signs of inflammation of the urinary and genital tracts.

Criteria for inclusion in the study were as follows:
- reproductive age;
- no pregnancy;
- the presence of complaints of discharge from the genital tract;
- irritation, burning in the vagina;
- frequent, painful urination;
- dyspareunia.

Exclusion criteria were as follows: pregnant women, patients with bleeding at the time of treatment due to benign hyperproliferative or malignant processes in the genital and urinary tract, children and postmenopausal age, the presence of cervical dysplasia.

All patients included in the study underwent gynecological examination, bacterioscopy and bacteriological exam-

ination with antibioticogram of vaginal and urethral secretions, oncocytology, colposcopy, PCR to detect chlamydia, gonococci, trichomonas, genital herpes, antipsychotics, cytomegalovirus, cytomegalovirus in accordance with the regulatory base adopted in Ukraine. Appropriate test systems were used in this research, such as: Euroimmun, Ampli-Sens, Tecan Sunrise (Germany). The premorbid background, the nature of menstrual function, the presence of a history of the pelvic organs inflammatory diseases and extragenital pathology were taken into account. The results obtained were processed by parametric statistics methods using the Excell software package adapted for biomedical research.

This study was carried out in compliance with ethical norms and rules adopted for biomedical research.

RESULTS

The composition of the urogenital microbiota of a healthy woman may have specific features dependent of the menstrual cycle phase and age [5, 6]. The optimal number and species composition of lactobacilli provides the acidity of vaginal secretions in the range of 4–4.5, the formation of a barrier for the fixation of opportunistic and pathogenic flora to vaginal epithelial cells, and also participates in a number of immune mechanisms [5, 7]. The composition of the microbiome is strongly correlated with the vaginal mucosa condition that depends primarily on the levels of sex hormones, including estrogen. The latter fact stimulates the proliferation of multilayered squamous epithelium and the production of glycogen in surface cells, which is a substrate for the lactobacilli activity [5, 8]. During studying the urogenital microflora of the above groups, the following pattern was observed: patients of the main group had a violation of the resident to facultative flora ratio (dysbiosis) on the background of borderline changes in the vaginal mucosa, mainly due to hormonal imbalance manifested by pathological vaginal discharge; instead, in the control group patients, the urogenital flora was saturated with a significant number of lactobacilli (90%). It is known that vaginal dysbiosis increases the likelihood of colonization by obligate-pathogenic flora. Recently, the data have been obtained on the possible influence of the dominance of certain species of facultative vaginal flora on the persistence of human papilloma virus and its carcinogenic potential [9, 10].

The age of the patients participated in the study was as follows: the main group with the average age of 24 ± 1.2 years; the control group with the average age of 23 ± 1.1 years. Patients of the main group visited the women's clinic on the 1st – 3rd day of disease (average number being 2.16 ± 0.08 days). From the anamnesis it is known that 32 (53%) patients of the main group had a burdened gynecological anamnesis and previously sought medical help in view of inflammatory diseases (colpitis, cervicitis, chronic salpingo-oophoritis, cystitis), compared with the control one, where the anamnesis was not burdened. When seeking medical advice, the patients of the main group complained of vaginal discharge – 56 (94%) women; itching, burning in the genital area – 58 (97%) persons; accelerated urination – 55 (92%) women; dysuria – 58 (97%) persons; pain associated with sexual intercourse – 60 (100%) patients. The main group patients, unlike the control group ones, had a following peculiarity: most of them 45 (75%) women were single with uncontrolled sexual intercourse and a large number of partners; 27 (90%) patients of the control group were married having one sexual partner; the socio-economic status of women in the main group was lower than that in the control group.

One should note the increased virulence of microorganisms, which are the trigger in the IPOD emergence. The spread of the infectious process can be facilitated by disordered sexual intercourse (with partners whose semen is the source of infection), intrauterine interventions, postpartum purulent-septic complications. The condition of women with this pathology is often influenced by so-called risk factors that not only cause exacerbation of the infectious process, but also are directly involved in the pathogenesis. These include unfavorable environmental situation in the place of residence (up to 12%), dangerous influence of certain occupational moments (8%), and bad habits (30–43%). It is known that various environmental factors, interpersonal relationships, violations of biological regimes can cause stress [8, 9].

When studying the premorbid background in 48 (80%) patients of the main group, there was a transferred bilateral salpingo-oophoritis with outpatient antibacterial, anti-inflammatory therapy, 17 (28%) patients with pelvic inflammatory disease suffered from the menstrual cycle violation of the dyspepsia type. In the control group patients, inflammatory pelvic organs (PO) processes, as well as violations of the ovarian-menstrual cycle, were not observed (normal menstrual function was preserved).

Bacterioscopic examination of vaginal discharge provided the following results: leukocytosis was detected in 54 (90%) patients of the main group, coccal and mixed microflora was observed in 35 (59%) women, specific pathogenic microflora was found in 21 (35%) persons. It should be noted that the specific pathogenic microflora of the vagina was represented by trichomonas in 4 (7%) patients, chlamydia – in 4 (7%) women, yeast cells and fungal spores – in 7 (12%) patients. Bacterioscopy of allocations in the control group patients has shown no specific features and demonstrated vagina normoflora in all cases.

Initial visual and colposcopic examination revealed hyperemia and edema of the vaginal mucosa in 60 (100%) patients of the main group, ectopia of the cylindrical epithelium was visualized in 25 (42%) cases, incomplete transformation zone – in 23 (38%) cases. Only the type II cytological picture in these women was observed for 60 patients/100%, whereas vaginal cleanliness of type III–IV was noticed. In the control group women, colpocytology showed no complaints and normal colposcopic pattern was observed.

Bacteriological examination of cervical canal and vaginal secretions in the examined patients from the main group revealed before treatment a combined nonspecific mixed

infection of the urogenital habitat against the background of lactobacilli microflora suppression. In all patients, the microbial landscape was represented by the gram-negative and gram-positive bacteria of different colonization degrees, i.e. *St. aureus* ≥105 CFU/ml – in 38 (63%) cases, *St. epidermidis* ≥105 CFU/ml – in 23 (38%) cases, *Bacteroides fragilis* ≥104 CFU/ml – in 17 (28%) cases, *Peptostreptococcus spp.* ≥104 CFU/ml – in 21 (35%) cases, *Escherichia coli* ≥104 CFU/ml – in 42 (70%) patients with a decrease in the number of *Lactobacillus spp.* The yeast-like fungi of the genus *Candida spp.* ≥104 CFU/ml were detected in 9 (15%) cases only. In the control group women, the urogenital microbiota was represented by a normal amount of *Lactobacillus spp.*

General analysis of urine in all patients of the main group (60 women) has shown leukocyturia (16–20 in field of view), squamous epithelium (10–12 in field of view), cylindruria (6–8 in field of view), mucus and bacteria (++), while the urine in the control group women (30 patients) was normal, with no pathology. Regarding urine culture in the main group, the following clinical pattern was observed: *E. coli* ≥ 105 CFU/ml – in 42 (70%) cases, *St aureus* ≥105 CFU/ml – in 32 (53%) cases, Klebsiella ≥ 106 CFU/ml – in 13 (22%) cases, *Enter. faecalis* ≥ 107 CFU/ml – in 10 (17%) women. In all the control group patients, the urine bacterial culture had no pathogenic microflora.

DISCUSSION

Inflammatory pelvic organs diseases (IPOD) are a hot problem in modern gynecology, as they occupy the first place in its structure (up to 70%), and also lead to serious long-term complications. Among them one should note infertility, chronic pelvic pain syndrome, ectopic pregnancy, miscarriage [9, 10]. The most noteworthy are urogenital infections, which are clinically manifested in the form of colpitis, cervicitis, chronic salpingo-oophoritis, cystitis, asymptomatic bacteriuria, chronic and gestational pyelonephritis, as well as extragenital infection foci that, in turn, cause complications and consequences unless detected or treated in proper time. After all, the biggest problem is the fact that in 80% of cases urogenital infection is latent and its presence in a woman may not be even suspected [1–3].

The urogenital microflora (microbiota) is a complex dynamic ecosystem. It includes normal/resident flora, which, in turn, is divided into the obligate and facultative ones. Under certain physiological conditions, the urogenital microbiota includes transient flora. Acquired flora can be represented by opportunistic pathogens [5]. The condition and composition of the vaginal microbiota is influenced by a large number of endogenous and exogenous factors that may or may not be modified. It is known that the dominant microorganisms of the vaginal microbiome are different species of lactobacilli (*Lactobacillus spp.*): *Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners, Lactobacillus jensenii*. Other species are normally present in much smaller quantities, i.e. *Bacteroides, Fusobacterium, Veillonela, Actinomycetes, Bifidobacterium, Peptococcus, Peptostreptococcus, Propionibacterium, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcal varicardia.*

When the microbiota of the above ecosystems in the vaginal biotope is disturbed, the concentration of bacteria of the genera Bacteroides, Fusobacterium, Peptostreptococcus, Clostridium, Eubacterium, Veillonella, Escherichia, Enterococcus, Mobiluncus, Gardnerella, Mycoplasma hominischialis increases, causing inflammatory urogenital process [1, 3, 7].

CONCLUSIONS

1. A high frequency of simultaneous lesions by the same pathogens of several anatomical areas, i.e. vagina, cervix, urethra, bladder, has been noted.
2. The above peculiarities require application of a multidisciplinary approach involving in treatment urologist and nephrologist.

The immune system of women with chronic urogenital infections in non-pregnancy status corresponds to the presence of an infectious process with changes at the systemic level. Such changes are the signs of an infectious process, they should be considered natural and cannot be considered as factors of infection, but only as its consequences and signs of specific or nonspecific inflammation.

REFERENCES

1. Radzynskyi V.E. Infektolohyia dvadtsat pervoho veka: neprostie otveti / Infektsyy i infektsyonnii kontrol [Infectious diseases of the XXI century: difficult answers / Infection and infection control]. Status Praesens. 2012; 2 (8):5-7. (In Russian).
2. Albota O. M., Berbets A. M. Mozhlyvist vykorystannia vahinalnoi tabletky mikronizovanoho prohesteronu pry bakterialnomu vahinozi u vahitnykh [The possibility of using a vaginal tablet of micronized progesterone in bacterial vaginosis in pregnant women]. Zhinochyi likar. 2016; 5 (67):48-50. (In Ukrainian).
3. Pasiechnikov S.P. Tsystyt: etiopatohenez, klasyfikatsiia, klinichna kartyna, diahnostyka, likuvannia [Cystitis: etiopathogenesis, classification, clinical picture, diagnosis, treatment]. Ukrainskyi Medychnyi Chasopys. 2016; 4 (114):34-37. (In Ukrainian).
4. Lakhno Y.V., Hryshchenko O.V. Stupenchataia terapyia vahynalnoho dysbyoza: mozayka dokazatelstv [Stepwise therapy for vaginal dysbiosis: a mosaic of evidence]. Reproduktyvna endokrynolohiia. 2016; 2 (28):58-59. (In Russian).
5. Yankovskyi D.S., Shyrobokov V.P., Antypkyn Yu.H. et al. Mykrobyom y zdorove zhenshchynы [Microbiome and women's health]. Reproduktyvna endokrynolohiia Almanakh reproduktyvnoho zdorovia. 2015:26-48. (In Russian).
6. Volodyna T.A., Saskova P.V., Yvanova E.V. Urohenytalnыi khlamydyoz: sovremennoe sostoianye voprosa (obzor) [Urogenital chlamydia: current state of the art (review)]. Farmatsyia y farmakolohyia. 2014; 6 (7):66-73. (In Russian).
7. Geerlings, S.E. Clinical Presentations and Epidemiology of Urinary Tract Infections. Microbiol Spectr. 2016; 4. doi: 10.1128/
8. Gupta K., Trautner B.W. Diagnosis and management of recurrent urinary tract infections in non-pregnant women. BMJ. 2013. doi: 10.1136.

9. Rossignol L., Vaux S., Maugat S. et al. Incidence of urinary tract infections and antibiotic resistance in the outpatient setting: a cross-sectional study (n.d.) Infection 45.1 (2017): 33-40. doi: 10.1007/s15010-016-0910-2.
10. Vovk I. B, Horban N. I, Kondratiuk V. K. et al. Optymizatsiia kompleksnoho likuvannia zapalnykh zakhvoriuvan henitalii u zhinok reproduktyvnoho viku [Optimization of complex treatment of inflammatory diseases of the genitals in women of reproductive age]. DU Instytut pediatrii, akusherstva i hinekolohii NAMN Ukrainy. Informatsiinyi lyst №8. 2015. (In Ukrainian).
11. Antonenko Y.V. Patohenetycheskye aspekты lechenyia vospalytelnыkh zabolevanyi vnutrennykh zhenskykh polovыkh orhanov na sovremennom эtape [Pathogenetic aspects of the treatment of inflammatory diseases of the internal female genital organs at the present stage]. Reproduktyvnaia endokrynolohyia. 2016; 4 (30):70-72. (In Russian).
12. Kohan B.H. Rol kombynyrovannыkh sredstv v kompleksnom lechenyy vospalytelnыkh zabolevanyi zhenskykh polovыkh orhanov smeshannoi atyolohyy [The role of combined agents in the complex treatment of inflammatory diseases of female genital organs of mixed etiology]. Zdorove zhenshchynы. 2014; 5 (91):86-90. (In Russian).
13. Maiorov M.V. Vospalytelnыe zabolevanyia orhanov maloho taza: rol kombynyrovannoi terapyy [Pelvic inflammatory disease: the role of combination therapy]. Medytsynskye aspekты zdorovia zhenshchynы. 2017; 2 (107):58-64. (In Russian).

ORCID and contributionship:
Nataliya Y. Bysaha: 0000-0001-6226-7294 [A,B]
Oxana O. Korchynska: 0000-0001-7265-4829 [C,D]
Stefania Andrascikova: 0000-0001-7960-6168 [E]
Silvia Zultakova: 0000-0003-0964-5748 [F]
Alena Schlosserova: 0000-0002-1747-1429 [C,E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Nataliya Y. Bysaha
Uzhgorod National University
1 Sq. Narodna, 88000 Uzhhorod, Ukraine
tel: + 380506715683
e-mail: your_natali@yahoo.com

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ORIGINAL ARTICLE

REPARATIVE REGENERATION OF PERIAPICAL TISSUES IN CHRONIC GRANULOMATOUS PERIODONTITIS UNDER THE INFLUENCE OF COMPOSITION BASED ON CALCIUM HYDROXYAPATITE

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Iryna V. Han, Volodymyr M. Zubachyk, Khrystyna H. Musij-Sementsiv, Nataliia I. Bodnaruk, Nataliia V. Porokhovska, Oksana M. Slaba

DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

The aim: To establish the dynamics of reparation processes in periapical tissues in chronic granulomatous periodontitis under the influence of the developed composition based on calcium hydroxyapatite in comparison with the generally accepted drug MTA.

Materials and methods: The main group included 52 individuals, and the comparison group – 19 patients with chronic granulomatous periodontitis. In the first experimental subgroup, patients were obturated of the tooth root apical area with the proposed composition, and in the second experimental subgroup patients were performed obturation of the root canal apical area with additional transcanal withdrawal of the composition into the periapical area. In the comparison group, the tooth root apex was obturated with MTA material.

Results: Clinical studies have shown that the composition based on calcium hydroxyapatite, having osteoconductive, antioxidant and anti-inflammatory properties, activates the synthesis of organic and mineral components, that promotes the bioreparation and regeneration processes of periapical tissues in chronic granulomatous periodontitis, which occurred most intensively during the first 6 months after treatment. At the same time, with additional transcanal removal of the filling composition based on calcium hydroxyapatite into the periapical area in patients with chronic granulomatous periodontitis with acquired wide tooth root apex was observed in 1.6 time better results than the method of creating an apical root canal obturation ($p < 0,05$), indicating a high therapeutic effect.

Conclusions: Composition based on calcium hydroxyapatite, having osteoconductive, antioxidant and anti-inflammatory properties, activates the synthesis of organic and mineral components, which promotes the processes of bioreparation and regeneration of periapical tissues, especially in the early stages. And additional transcanal removal of the filling composition into the periapical space causes the acceleration of the period of periodontal tissues revitalization, bioreparation and regeneration compared to the creation of the apical obturation within the physiological tooth root apex.

KEY WORDS: granulomatous periodontitis; composition based on calcium hydroxyapatite; regeneration; CDAP index

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INTRODUCTION

The bone tissue regeneration after its damage by various factors remains an urgent problem, both for medicine in general and for dentistry in particular [1-4]. With the latest technologies and inventions, the prevalence of complicated caries is not only not reduced, but, according to some authors, increases, which is associated with the complexity of endodontic treatment and quite often – with the absence of long-lasting positive results after its completion [5-10].

In the conservative treatment of patients with chronic forms of periodontitis, the main tasks remain the influence on the microflora of macro- and microchannels with the aim of their sterilization, hermetic root canals obstruction to prevent root canal reinfection and direct influence on the periapical tissues with the aim of stimulation of regenerative processes in the area beyond the apex, both by enhancing the body's defenses and by influencing the components of the endodontic filling material to accelerate reparative processes in the periapical area [11-15].

Thus, the high prevalence of chronic forms of periodontitis causes the constant improvement of already developed types of endodontic medicine and the search for new means and methods for tight obturation of the tooth root apex and stimulation of the revitalization and regeneration of periapical tissues [16, 17].

THE AIM

To establish the dynamics of reparation processes in periapical tissues in chronic granulomatous periodontitis under the influence of the developed composition based on calcium hydroxyapatite in comparison with the generally accepted drug MTA.

MATERIALS AND METHODS

71 people aged 20 to 59 years, who were suffering from chronic granulomatous periodontitis, were under our observation. All teeth were single-rooted with the width of the root apex 40 to 80 per ISO. Patients provided voluntary written consent to conduct a clinical trial. The study was confirmed and approved by the ethics committee of Danylo Halytsky Lviv National Medical University.

The patients were divided into two groups: the main group consisted of 52 individuals and the comparison group – 19 patients. The main group, according to the technique of root canal filling, was divided into two subgroups: patients in the first experimental subgroup were obturated apical section of the tooth root by our proposed composition containing calcium hydroxyapatite, a means for revitalization and bioreparation of periapical tissues, 40% polyvinylpyrrolidone and barium sulfate (patent of Ukraine № 95974) by creation the apical obturation (patent of Ukraine № 95967) [18, 19]; patients in the second experimental subgroup were obturated the apical section of the root canal with additional transcanal removal of the composition into the periapical area.

The condition of periapical tissues was evaluated on the basis of complaints, anamnesis, objective data and an additional examination method – radiography [20, 21, 22]. Patients were made intra-oral radiography of the teeth during the initial examination, after completion of endodontic treatment and at the stages of dispensary observation in the control periods: 6, 12 and 18 months. The quality of root canal obturation was evaluated and the magnitude of the destruction of changes beyond the apex was diagnosed using the periapical index of the condition of destruction of apical periodontitis (CDAP) in scores E.O. Skapkareva, 2003 [23, 24].

Patients with chronic granulomatous periodontitis of the comparison group after antiseptic treatment of the oral cavity were performed carious cavity preparation and tooth cavity opening, thereafter an access to root canals was created, the tissue breakdown products were evacuated, the length of the root canal was measured by apexlocator using a K-file and X-ray method. Mechanical cleansing and extension of the root canal were performed by the "Crown down" technique using an endomotor. The crown part of the root canal was worked out by Shaper endodontic instruments, and ProFile instruments of various sizes and tapers were used for the middle and the apical part of the root canal, not reaching the anatomical tooth root apex by 3-4 mm. The apex part of the root canal was worked out with hand-held K-and H-file type endodontic instruments with sizes from № 40 to № 80 according to ISO using the "RC-prep" endogel ("Premier Dental", USA) for root canal. Instrumental processing of the root canals was completed at the level of the anatomical apex of the root canal under the control of the apexlocator and radiographic method. Medical root canal treatment was performed using endodontic syringes with irrigation solutions, which included 3% hydrogen peroxide solution, 3% and 5,25% sodium hypochlorite solution and distilled water. After drying, the root canal was filled with calcium-containing paste «Calasept» («Nordiska Dental», Sweden) and a temporary seal was applied. At the second visit, after repeated antibacterial and instrumental treatment of the canal, the root apex was covered with MTA material ("Cercamed", Poland) and a temporary seal was applied. At the third visit, the canal from the apical obturation level and to the root canal orifices was sealed by central pin method or lateral condensation method with AH Plus material («Dentsply», USA) in combination with gutta-percha pins. Thereafter, the anatomical shape of the crown part of the tooth was restored with photopolymeric light solidification materials. X-ray examination was performed during the initial examination, after completion of endodontic treatment and at the stages of dispensary observation in the central pin or cold lateral condensation with the restoration in control dates.

Treatment of patients with chronic granulomatous periodontitis of the main group was carried out according to the basic method. After the opening of the tooth cavity, evacuation of putrid masses, mechanical expansion and antiseptic treatment of the root canal with the purpose of prolonged impact on the periapical tissues, a paper pin, soaked in the means for revitalization and bioreparation, was left inside. The solution contained hyaluronic acid modified with vitamin C and amino acids – proline, lysine and glycine, as well as 1% collagen hydrolyzate, 6% 2-dimethylaminomethanol 4-acetoaminobenzoate and 1% organic silicon in equal proportions of components (patent of Ukraine № 85259). For the purpose of obturation of the apical part of the root canal (patent of Ukraine № 95967) and the removal of the therapeutic agent in the area beyond apex, a composition in the form of a medicinal paste was developed, which composition included the ingredients mentioned above and calcium hydroxyapatite as well as excipients: polyvinylpyrrolidone and barium sulfate (patent of Ukraine № 95974) [25]. For the apical obturation of the root canal by the composition, plaggers of appropriate sizes were used, and the removal of the composition was transcanal carried out using channel fillers "Lentulo" 30-40 sizes. A cotton ball was placed on the root canal orifices and the tooth was closed with a temporary seal for 1-2 days. In the subsequent visit, the canal from the apical obturation level and to the root canal orifices was sealed by the method of anatomical tooth shape with light solidification composite materials.

RESULTS

During the examination of patients with chronic granulomatous periodontitis, it was found that only two patients in the main group (3,9%) had a complaint of minor pain while eating immediately after treatment (Table 1). By the second or third day, all signs of inflammation had gone away without further treatment. In the other patients – 52 persons (96,1%) of the experimental group, we observed restoration of tooth function, which testifies to the positive dynamics of their treatment.

Table I. Criteria for the effectiveness of treatment of patients with chronic granulomatous periodontitis immediately after its conduct

Examination groups		Complaints		Pain during percussion		Mucous edema in root apex projection		Restoration of tooth function	
		yes	no	yes	no	yes	No	yes	No
Comparison group, n = 19	abs.	5	14	5	14	4	15	14	5
	%	26,3	73,7	26,3	73,7	21,1	78,9	73,7	26,3
	the average indicator in the group	0,26±0,1		0,26±0,1		0,21±0,1		0,26±0,1	
The first experimental subgroup, n = 28	abs.	1	27	1	27	0	28	27	1
	%	3,6	96,4	3,6	96,4	0	100	96,4	3,6
	the average indicator in the group	0,06±0,04		0,06±0,04		0		0,06±0,04	
The second experimental subgroup, n = 24	abs.	1	23	1	23	0	24	23	1
	%	4,2	95,8	4,2	95,8	0	100	95,8	4,2
	the average indicator in the group	0,04±0,04*		0,04±0,04		0		0,04±0,04	

Note: * - the probability indicator of difference between the average indicators compared to the comparison group at p <0,05

Table II. Criteria for the effectiveness of treatment of patients with chronic granulomatous periodontitis after 6 months

Comparison groups		Complaints in anamnesis		Pain during percussion		Restoration of tooth function		The CDAP index
		yes	no	yes	no	yes	No	
Comparison group, n = 19	abs.	4	15	4	15	15	4	3,9±0,27
	%	21	79	21	79	79	21	
	the average indicator in the group	0,21±0,1		0,21±0,1		0,21±0,1		
The first subgroup of the main group, n = 28	abs.	1	27	1	27	27	1	3,2±0,16*
	%	4	96	4	96	96	4	
	the average indicator in the group	0,04±0,04		0,04±0,04		0,04±0,04		
The second subgroup of the main group, n = 24	abs.	0	24	0	24	24	0	2,8±0,17***
	%	0	100	0	100	100	0	
	the average indicator in the group	0		0		0		

Note: * - the probability indicator of difference compared to the comparison group at p <0,05; *** - at p <0,001.

In the comparison group, complaints of pain during tingling and positive percussion of the causative tooth immediately after the treatment were observed in 5 patients (21%), apart from this, 3 of them (10,7%) showed swelling in the transient convolution of the oral mucosa in the area of the oral cavity in the tooth root apex and tenderness during its palpation. All patients with exacerbation of the inflammatory process were prescribed the drug "Nurofen". After conducting of additional treatment for ten days, all signs of exacerbation of the inflammatory process were eliminated.

At the same time, the results of the studies indicate that the index of CDAP in patients with chronic granulomatous periodontitis of the main group both of the first and second subgroups was 4,3 ± 0,18 point, and in the comparison group – 4,4 ± 0,23 point, that indicates on the primary approximately the same severity of destruction processes in periapial tissues in patients with chronic granulomatous periodontitis.

The effectiveness of treatment largely depends not only on the means, but also the method used in the treatment of this pathology. During the control examination of patients with chronic granulomatous periodontitis with acquired tooth root apex, it was observed the maintenance of functional activity of the tooth in 96,4% and 100% of patients 6 months after the endodontic treatment in the first and second subgroups of the main group. Only one patient (3,6%) of the first subgroup complained of minor periodic discomfort when biting a tooth. In the comparison group, the preserved functional tooth activity was noted in 79% of patients, which is 20% less than in the main group. This

Table III. Meaning of the CDAP index indicators (points) of patients with chronic granulomatous periodontitis 12 months after treatment

Terms of examination	Examination groups		
	Comparison group, n= 19	Main group, subgroup 1, n= 28	Main group, subgroup 2, n= 24
6 months	3,9 ± 0,27	3,2 ± 0,16	2,8 ± 0,17
12 months	3,4 ± 0,31**	2,4 ± 0,19*** °°	1,7 ± 0,2 *** °°° ◊

Note: ** - the probability indicator of difference compared to the results on 6 month of the study at p <0,01; *** - at p <0,001; ° - the probability indicator of difference compared to the comparison group at p <0,05; °°° - at p <0,001; ◊ - the probability indicator of difference between the subgroups of the main group at p<0,05.

Table IV. Meaning of the CDAP index indicators (points) of patients with chronic granulomatous periodontitis 18 months after treatment

Terms of examination	Examination groups		
	Comparison group, n= 19	Main group, subgroup 1, n= 28	Main group, subgroup 2, n= 24
12 months	3,4 ± 0,31	2,4 ± 0,19	1,7 ± 0,2
18 months	2,4 ± 0,42***	1,3 ± 0,19*** °	0,8 ± 0,17 *** °°°

Note: *** - the probability indicator of difference compared to the group immediately after treatment at p <0,001; ° - the probability indicator of difference compared to the comparison group at p <0,05; °°° at - p <0,001.

is due to the presence of complaints in the anamnesis of 4 patients, which was 21%. At the same time, the pain and edema were not detected and percussion of the causative tooth was not painful at the time of examination and palpation of the mucous membrane in the area of the tooth root apex.

In all patients of the main group with chronic granulomatous periodontitis there was observed a decrease in the area of destruction of bone tissue and its replacement on the edges of lesions with a healthy bone tissue on radiographs after 6 months after the treatment (Table 2). It was also noted a decrease in the volume of excretion of the filling composition into the extracellular space in patients of the second subgroup. Instead, in the comparison group, only 2 patients (10,5%) had an increase in the area of the lesion beyond the apex, and the lesion of 5 people (26%) remained unchanged. Thus, the analysis of the results shows that only 63% of patients in the comparison group had a positive dynamics after conducted treatment, which was 37% less than the main group. At the same time, the average indicator of the CDAP index in the first subgroup of the main group was 3,2 ± 0,16 point, in the second subgroup – 2,8 ± 0,17 point, and in the comparison group, the average index was 3,9 ± 0,27 point . The results indicate that it was 1,2 time and 1,4 time lower in the first and second subgroups, respectively, compared to the control group and statistically significant (p <0,001).

After 12 months after treatment, analyzing the results of clinical examination of patients with chronic granulomatous periodontitis, it was established the preserved functional activity of the tooth in 100% of patients in the main group, and in the comparison group only in 84% of people (16 patients) (Table 3). During the X-ray study, all patients of the main group were observed a further reduction of the bone thinning area and replacement it with a newly formed bone, and patients of the second subgroup of the main group were noted the further integration of the components of the composition in the regeneration processes. However, the positive dynamics in the comparison group was observed only in 84% of patients, and in 2 patients (10,5%) we found an increase in the size of destruction of changes beyond the apex, and in one patient (5,3%) – it remained unchanged. In the first subgroup, the average index of the CDAP index decreased by 0,8 point and became 2.4 ± 0.19 point, and in the second subgroup – by 1,1 point and became 1,7 ± 0,2 point (p <0,05). At the same time, in the comparison group it was decreased by only 0,5 point and became 3,4 ± 0,31 point (p <0,01). In 11% of patients in the comparison group, the CDAP index increased or remained unchanged, indicating that there was no positive dynamics. However, in all patients of the main group, the CDAP index decreased, indicating 100% positive dynamics of regeneration processes in the periapical zone, and the fact that method of additional transcanal filling creates the favorable conditions for reducing the time of regeneration.

During the clinical examination of patients with chronic granulomatous periodontitis with acquired wide tooth root apex, it was observed in 100% of cases the preserved functional activity of the tooth in the main group after 18 months after treatment, and in the comparison group in 84% of people (16 patients), (Table 4). During the analysis of the results of radiological examination in the treatment by the method of creating apical obturation with the developed composition, complete recovery of periapical tissues was noted in 8 people (28.6%), and with additional transcanal removal of this composition into the periapical space – in 11 patients (46%). However, in the comparison group, restoration of periapical tooth tissues was observed in only 3 patients, which amounted to 15,7%, and in 5

patients (18%) the CDAP index remained unchanged. In the first experimental subgroup it decreased by 0,9 point and was 1,3 ± 0,19 point, and in the second subgroup by 1,1 point – 0,8 ± 0,17 point, which is statistically significant ($p < 0,001$) . In the comparison group, the average index decreased by 1,0 point ($p < 0,001$). The structure and volume of the root canal obturation was preserved in all patients and at all stages of the patient monitoring.

DISCUSSION

In our opinion, the significantly lower percentage of exacerbations in patients of the main group is due to the influence of the composition components having anti-inflammatory and antioxidant effect on the periapical tissues of the tooth [22, 24].

The effectiveness of treatment largely depends not only on the means, but also the method used in the treatment of this pathology. The decrease in the area of bone tissue destruction and its replacement at the edges of the lesion with the healthy bone tissue in persons of the first subgroup, as well as the reduction of the volume of the filling composition withdrawn into the area beyond the apex in persons of the second subgroup of the main group testify to the ability of the composition to biodestruction under conditions of destructively altered periodontal tissues and inclusion of its components in the regenerative process of the new bone tissue formation [15,16].

It should be noted that provoding the treatment of chronic granulomatous periodontitis with the use of the proposed composition in the examination after 6 months it was obtained better results than the general method of treatment using the drug MTA. In our opinion, such a positive result of treatment of the patients of the main group is associated with the anti-inflammatory, osteotropic and antioxidant properties of the composition based on HA, which promoted acceleration of the regeneration processes. At the same time, it was noted the direct dependence of the treatment results in the main group on the method of filling the root canals, which was more effective in case of withdrawal of the composition into the periapical space [9, 11, 17].

According to clinical and radiological studies of patients 12 months after treatment, we can state that the frequency of exacerbation cases of the inflammatory process in patients with chronic granulomatous periodontitis is higher when sealing with the drug MTA compared to the proposed composition. And the speed of the regeneration process is better when applying a composition based on calcium hydroxyapatite compared to the generally accepted drug MTA. In 11% of patients in the comparison group, the CDAP index increased or remained unchanged, indicating that there was no positive trend. At the same time, the CDAP index decreased in all patients of the main group, indicating 100% positive dynamics of regeneration processes in the periapical zone, and the method of additional transcannal filling creates favorable conditions for reducing the time of regeneration [10, 24].

The results of studies throughout the observation period for patients with chronic granulomatous periodontitis showed that the intensity of the regeneration processes was quite high with the highest activity during the first 6 months after treatment. Additional transcanal removal of the composition into the area beyond the apex was 1,6 time more effective after 18 months compared to the group where the treatment was performed by the method of apical obturation ($p < 0,05$) [17].

Summarizing the results of the study, it can be argued that the composition based on calcium hydroxyapatite furthers the acceleration of the regeneration processes in the periapical area and the reduction of the recovery time of patients with granulomatous periodontitis. In particular, the results of the study indicate that the most intensive regeneration processes in patients of granulomatous periodontitis of the main group took place during the first 6 months of observation after treatment. In our opinion, this is due to the influence of organic components of the composition, which during this period are more intensively releasing from the dosage form and stimulate bioregeneration processes and exhibit anti-inflammatory properties. At a stage of 12-18 months, a similar trend remains, which is due to the osteoconductive properties of hydroxyapatite [7, 15, 16].

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CONCLUSIONS

1. Composition based on calcium hydroxyapatite, having osteoconductive, antioxidant and anti-inflammatory properties, activates the synthesis of organic and mineral components, which furthers the processes of bioreparation and regeneration of periapical tissues in chronic granulomatous periodontitis, especially in the early stages.
2. Additional trans-channel withdrawal of the filling composition into the periapical area causes the acceleration of the revitalization, bioreparation and regeneration periods of periodontal tissues compared to the creation of an apical obturation within the physiological apex of the tooth root, which provides a high therapeutic effect.
3. For the treatment of patients with chronic granulomatous periodontitis, especially with the acquired apex of the root of the tooth, in order to accelerate the repair processes of the tissue beyond the apex, it is possible to recommend the proposed osteotropic composition based on calcium hydroxyapatite, which will stimulate the biorevitalization and reparation of tissues beyond the apex.

REFERENCES

1. Noor E., AL-Bayaty F. A review on predisposing and modifying factors of periodontal disease. Journal of Advanced Medical Research. 2015; 5(1):5–23.
2. Louis M., George T., Huang J. Regeneration and Repair in Endodontics. A Special Issue of the Dentistry Journal. Dent J. 2015; 3(3):77–78.
3. Saoud T., Ricucci D., Lin L. Regeneration and repair in endodontics – a special issue of the regenerative endodontics – a new era in clinical endodontics. Dentistry Journal. 2016; 4(1):1–15.

4. Ziętek M., Skrzypczyk I., Szulc M. Comparative analysis and mutual relations between mariginal periodontitis and periapical lesions. literature review. Dent. Med. Probl. 2015; 52(3):265–268.
5. Singh O., Sinha D., Tyagi S., Tyagi N. Root resorption: challenge to the endodontist. International Jornal of Dental Research and Oral Sciences. 2017; 2(1):31–41.
6. Garcia C., Khouly I., Sanz J., Loomer P. Effectiveness of systemic antimicrobial therapy in combination with scaling and root planning in the treatment of periodontitis : a systematic review. J. Amer. Dent. Assos. 2015; 146(3):150–163.
7. Sculean A. , Nikolidakis D., Nikou G., Biomaterials for promoting periodontal regeneration in human intrabony defects: a systematic review. Periodontology 2000. 2015; 68(1):182—216.
8. Bansal R., Jain A., Mittal S. Current overview on challenges in regenerative endodontics. J. Conserv Dent. 2015;18:1—6.
9. Bartols A., Roussa E., Walther W. First Evidence for Regeneration of the Periodontium to Mineral Trioxide Aggregate in Human Teeth. JOE. 2017; 43(5):715–722.
10. Saoud T., Martin G., Chen Y. Treatment of mature permanent teeth with necrotic pulps and apical periodontitis using regenerative endodontic procedures: A case series. J. Endod. 2016;42(1):57–65.
11. Körklü S., Candaner Y., Özçelik E. Treatment of permanent teeth with extensive apical periodontitis: 2 case reports. British Journal of Medicine and Medical Research. 2016; 17(10):1–7.
12. Saoud T., Huang G., Gibbs J. Management of Teeth with Persistent Apical Periodontitis after Root Canal Treatment Using Regenerative Endodontic Therapy. J. Endod. 2015; 41(10):1743–1748.
13. Kotwal G., Sarojini H., Chien S. Pivotal role of ATP in macrophages fast tracking wound repair and regeneration. Wound Repair Regen, 2015; 23(5):724–727.
14. Khoshbin E., Donyavi Z., Atibeh E. Effect of Nd:YAG and Diode Lasers on Apical Seal of Root Canals Filled with AH Plus and Mineral Trioxide Aggregate-Based Sealers. J Dent (Tehran). 2018; 15(1):30–40.
15. Sokhi R., Sumanthini M., Shenoy V. Effect of Calcium Hydroxide Based Intracanal Medicaments on the Apical Sealing Ability of Resin Based Sealer and Guttapercha Obturated Root Canals. J Clin Diagn. 2017: 11(1):75–79.
16. Zubachyk V., Han I. Comparative evaluation of osteotropic action of endodontic filling materials on the model of experimental destruction of rats' jaw bone tissue . Ukrainian Dental Almanac. 2016;1(2):12–16.
17. Han I., Musij-Sementsiv K., Zubachyk V. The effect of endodontic filling agents on the activity of the regeneration processes of bone tissue in the experiment. JMS. 2018; 87(1):11–18.
18. Zubachyk V.M., Han I.V. Composition for the treatment of complicated caries of permanent teeth with wide foramen apex and at the stage of root formation Pat. 95974, A61K 33/00, A61K 33/06. № u 201408800; appl. 04.08.2014; publ. 12.01.15, Bul. №1.
19. Zubachyk V.M., Han I.V. Pat. 95967, A61C 5/00, A61P 1/02. The method of treatment of complicated caries of permanent teeth with wide foramen apex and at the stage of root formation / № u 201408737; appl. 01.08.2014; publ. 12.01.15, Bul. №1.
20. Kanagasingam S., Lim C., Yong C. et al. Diagnostic accuracy of periapical radiography and cone beam computed tomography in detecting apical periodontitis using histopathological ndings as a deference standard. Int Endod J. 2017; 50(5):417–426.
21. Filho E., Calisto A., Tavarez R. Correlation between the Periapical Index and Lesion Volume in Cone-beam Computed Tomography Images. Iranian Endodontic Journal. 2018; 13(2):155—158.
22. Ricucci D., Siqueira J., Loghin S. Repair of extensive apical root resorption associated with apical periodontitis: Radiographic and histologic observation after 25 years. J. Endod. 2014; 40(8):1268–1274.
23. Orstavic D., Kerekes K., Eriksen M. The periapical index: a scoring system for radiographic assessment of appical periodontitis. Endodontic dental Traumatol, 1986; 2:20–34.
24. Filho E., Calisto A., Tavarez R. Correlation between the Periapical Index and Lesion Volume in Cone-beam Computed Tomography Images. Iranian Endodontic Journal. 2018; 13(2):155—158.

ORCID and contributionship:
Iryna V. Han: 0000-0003-2605-5029 [A,C,F]
Volodymyr M. Zubachyk: 0000-0003-3813-5114 [B,D]
Khrystyna H. Musij-Sementsiv: 0000-0003-3068-8611 [F,D]
Nataliia I. Bodnaruk: 0000-0002-5222-7915 [E,F]
Nataliia V. Porokhovska: 0000-0001-5221-8544 [C]
Oksana M. Slaba: 0000-0003-4566-7178 [E]

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CORRESPONDING AUTHOR
Iryna V. Han
Danylo Halytsky Lviv National Medical University
69 Pekarska St., 79010 Lviv, Ukraine
tel:+380676733304
e-mail: irynagan@gmail.com

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ORIGINAL ARTICLE

OBTURATION INTESTINAL OBSTRUCTION IN THE COURSE OF NECROTIZING ENTEROCOLITIS IN NEWBORN CHILDREN

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Olga M. Gorbatyuk[1], Stanislav I. Babuci[2], Taras V. Martinyuk[3], Konstantin T. Bertsun[4]
[1]SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KYIV, UKRAINE
[2]INSTITUTE OF MOTHER AND CHILD, CHISINAU, REPUBLIC OF MOLDOVA
[3]VOLYN REGIONAL TERRITORIAL MEDICAL ASSOCIATION FOR MATERNAL AND CHILD WELFARE, LUTSK, UKRAINE
[4]VINNYTSIA REGIONAL CHILDREN'S CLINICAL HOSPITAL, VINNYTSIA, UKRAINE

ABSTRACT

The aim: To analyze our own results of diagnosis and treatment of newborns with NEC and obturation intestinal obstruction in order to determine theoretically important and practically significant recommendations on this issue, to optimize preventive measures for reducing intestinal obstruction cases in NEC and improving treatment outcome among newborns.

Materials and methods: Our supervision involved 143 newborns with NEC for the period from 2006 to 2020, including 79 boys (55.24%) and 64 girls (44.76%). Gestational age was 26-42 weeks. The majority of children were premature infants and numbered 121 children (84,62%). The assessment of clinical manifestations, progression of the disease, physical examination of newborns were used to help in diagnosing. All children underwent general clinical tests and instrumental methods of diagnosis including ultrasonography (abdominal organs and retroperitoneum, echocardiography, neurosonography) and X-ray examination (two-dimensional plain abdominal radiography, X-ray contrast study of the gastrointestinal tract). According to the survey, all patients were examined by allied health professionals.

Results: Obturation intestinal obstruction associated with NEC was diagnosed in 26 children (18.18%). 12 children were in serious condition, representing 46.15%. The condition of other 14 children (53.85%) was assessed as critical one. The first signs of intestinal obstruction were observed in children with NEC at the end of the first week or at the beginning of the second week of life. Intestinal obstruction was acute in 21 children (80.77%); 5 children (19.23%) had relapsing course. Blood test results showed the inflammatory process and were not specific. According to the results of our observation, the dynamics of platelets is indicative as there is a correlation between the deterioration in the child's condition and the onset of symptoms of intestinal obstruction and thrombocytopenia. There was an imbalance in protein and electrolyte metabolism – a decrease in the level of albumin, indicators of K, Na, Ca, and an increase of C-reactive protein by several times. Ultrasonography of the abdominal organs showed irregular intestinal pneumatization, dilated intestinal loops with stagnant intestinal contents, pendulum peristalsis, infiltrative bowel wall thickening, free fluid in the abdominal cavity. Plain radiography revealed typical manifestations of small intestinal obstruction with multiple air-fluid levels. In 15 newborns (57.69%), conservative treatment was effective, in particular intestinal obstruction was relieved; 11 newborns (42.31%) underwent surgery. The mortality was 36.36% (4 children died). Postoperative complication in the form of bowel stenosis in the area of the direct anastomosis was observed in 3 patients approximately 2 weeks after the surgery.

Conclusions: 1. NEC can lead to the development of obturation intestinal obstruction in newborns. Obturation intestinal obstruction was a form of NEC course in 18.18% of children under our observation.

2. Obturation intestinal obstruction in NEC in newborns is a reversible condition and requires conservative therapy. If symptoms of obstruction persist within 3 days after the start of conservative therapy, surgical treatment is indicated.

3. In case of obturation intestinal obstruction, it is advisable to perform resection of necrotic bowel, formation of a double enterostomy.

4. Enterostomy closure by placing T-shaped intestinal anastomosis should be performed 4 weeks after the primary surgery.

5. The use of modern techniques for early diagnosis and timely treatment of obturation intestinal obstruction in NEC can reduce mortality and the number of postoperative complications.

6. Newborns with NEC should be under the supervision of surgeons, which will help timely determine the indications for surgical intervention.

KEY WORDS: necrotizing enterocolitis, newborns, obturation intestinal obstruction,treatment

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INTRODUCTION

Obturation intestinal obstruction is a partial or complete occlusion of the intestinal lumen as a result of its blockage, external compression, or the presence of pathological process in the wall, which closes the lumen of the intestinal tube. Necrotizing enterocolitis (NEC) in newborns is a multifactorial disease which occurs as a result of severe intestinal lesion due to combined effect of potentially harmful factors and is characterized by acute inflammation of the bowel wall, dissection of gas into the bowel wall as a result of invasion of intestinal gas-forming microorganisms, ischemia and possible necrosis [1, 2, 3].

Despite numerous epidemiological studies, the true incidence of NEC among newborns is still contradictory. Thus,

Table I. Distribution of newborns with NEC by their number and body weight

Body weight (g)	Number of children (n)	Number of children (%)
500 – 1000	12	8.39
1001 – 1500	46	32.17
1501 – 2000	45	31.47
2001 – 2500	22	15.38
2501 – 3000	11	7.69
3001 – 3500	4	2.80
greater than 3500	3	2.00
Overall total	143	100

according to the National Institute of Child Health and Human Development (the USA), the incidence of NEC in 2003-2007 is reported to be 11% among premature infants with a body weight of less than 1500 g [4]. Studies of the Canadian Neonatal Network in 2003-2008 showed that the incidence of NEC was 5.1% [5], and the Swiss research reported 3-4% among children less than 32 weeks of gestational age [6].

Prematurity and low birth weight are traditionally considered to be important risk factors for the development of NEC and its complications. Recent studies have shown differences in bacterial colonization, microcirculatory perfusion, maturity of gastrointestinal immunity in full-term and premature infants and these differences explain the development of obturation intestinal obstruction mainly in premature infants [7, 8, 9].

The course of NEC may vary from nonspecific symptoms of mild local bowel lesions to systemic advanced disorders with the development of multi organ failure [10, 11]. The progression of NEC and the development of surgical stages of the disease may occur within a few hours to 8 days from the disease onset. NEC can further lead to the development of obturation intestinal obstruction and cicatrical stenosis of the intestine. The determinants of obturation intestinal obstruction in children with NEC involve intestinal hypomotility, lack of digestive enzymes, hardening of the small bowel contents, luminal obstruction of the ileum. There are three types of obturation intestinal obstruction: intraorganic, intramural (cicatricial strictures), and extraorganic, which occurs as a result of a complete or partial extrinsic blockage of the intestinal lumen, without disturbed circulation. In newborns who required surgical interventions for intestinal obstruction, systemic manifestations of NEC prevailed over local ones, and the general condition worsened from severe to very severe [12].

In recent years, there has been a trend towards an increase in the incidence of obturation intestinal obstruction associated with NEC [13]. Treatment success depends on early diagnosis of the pathology and adequate treatment policy. Today, there is no consensus on the method of creating an anastomosis in small intestinal obstruction in newborns with NEC, which determines the relevance of this problem [15].

THE AIM

The aim of the paper is to analyze our own results of diagnosis and treatment of newborns with obturation intestinal obstruction associated with NEC in order to determine theoretically important and practically significant recommendations on this issue, to optimize diagnostic, therapeutic, preventive measures for reducing intestinal obstruction cases in NEC and improving treatment outcome among newborns.

MATERIALS AND METHODS

Our supervision involved 143 newborns with NEC for the period from 2006 to 2020 (67 children at Volyn Regional Children's Territorial Medical Association for Maternal and Child Welfare and 76 children at Vinnytsia Regional Children's Clinical Hospital). There were 79 boys (55.24%) and 64 girls (44.76%) among them. Gestational age was 26-42 weeks. The total number of patients with NEC in absolute numbers and as a percentage of body weight is shown in Table 1.

A child with minimum body weight was 580 g at the age of 26 weeks; the maximum body weight among children was 3880 g. The majority of children were premature infants and numbered 121 children (84.62%).

The assessment of clinical manifestations, progression of the disease, physical examination of newborns (examination, abdominal palpation and auscultation) were used to help in diagnosing. All children underwent general clinical tests (clinical blood and urine analysis, blood glucose test, blood group and Rh factor, protein electrophoresis, blood electrolytes, studies of urea, creatinine, total bilirubin and its fractions, ALT, AST, C-reactive protein) and instrumental methods of diagnosis including ultrasonography (abdominal organs and retroperitoneum, echocardiography, neurosonography) and X-ray examination (two-dimensional plain abdominal radiography, X-ray contrast study of the gastrointestinal tract). According to the survey, all patients were examined by allied health professionals.

The study is carried out as part of research project of the Department of Pediatric Surgery of Shupyk National Medical Academy of Postgraduate Education entitled "Development Of New Treatment Methods And Their Assessment In Congenital and Acquired Surgical Pathology In Children" (state registration number 0118U003564).

Nonparametric statistical methods of processing medical and biological research were used in the work due to the small number of the sample and the predominance of analysis of qualitative rather than quantitative features. Numerical data are presented in absolute values (n) and percentage (%).

RESULTS

Obturation intestinal obstruction associated with NEC was diagnosed in 26 children (18.18%), including:
- 4 patients with a body weight of less than 1000 g,
- 15 newborns with a body weight of 1001-1500 g,
- 5 children with a body weight of 1501-2500 g,
- 2 children with a body weight of over 2500 g.

The main signs of obturation intestinal obstruction involved frequent regurgitation and vomiting bile or intestinal contents, increased abdominal distention, anterior abdominal wall oedema, intestinal loop contouring, crepitus in dilated intestinal loops on palpation, gas and stool retention. The first signs of intestinal obstruction were observed in children with NEC at the end of the first week or at the beginning of the second week of life. Intestinal obstruction was acute in 21 children (80.77%); 5 children (19.23%) had relapsing course.

Abdominal distension, asymmetry, apnea were observed during abdominal examination. Abdominal palpation revealed local or general abdominal guarding, local or general tenderness, fluid-filled intestinal loops, pathological formations in the abdominal cavity. On auscultation, peristalsis was mostly weakened or absent.

Blood test results showed the inflammatory process and were not specific. According to the results of our observation, the dynamics of platelets is indicative as there is a correlation between the deterioration in the child's condition and the onset of symptoms of intestinal obstruction and thrombocytopenia. There was an imbalance in protein and electrolyte metabolism – a decrease in the level of albumin, indicators of K, Na, Ca, and an increase of C-reactive protein by several times.

Ultrasonography of the abdominal organs showed irregular intestinal pneumatization, dilated intestinal loops with stagnant intestinal contents, pendulum peristalsis, infiltrative bowel wall thickening, free fluid in the abdominal cavity.

X-ray examination revealed typical manifestations of small intestinal obstruction with multiple air-fluid levels; in 2 children the obstruction was in the large intestine.

Obturation intestinal obstruction in the severe course of NEC refers to emergency neonatal surgery and can be managed conservatively or require surgical intervention.

Conservative treatment is carried out within 3 days with obligatory gastrointestinal decompression. No stimulating medicamentous therapy. In 15 newborns (57.69%), conservative treatment was effective, in particular intestinal obstruction was relieved, passage of flatus and bowel movements resumed.

The scope of conservative therapy:
1. Cessation of enteral feeding. Total intravenous nutrition
2. Gastrointestinal decompression (washing at least 3 times a day)
3. Antibiotic therapy (Metronidazole, Amikacin, the third- and fourth-generation Cefalosporins)
4. Infusion therapy
5. Immune therapy (Biovenum Mono, Octagam)
6. Enzyme therapy and eubiotics.

Eleven (42.31%) out of 26 patients with intestinal obstruction underwent surgery. Among newborns who underwent surgery, in 7 children intestinal obstruction was localized in ileum, in 2 children – in jejunum, in 2 children – in large intestine.

The scope of surgical interventions consisted of the following:
- intestinal exploration, adheziolysis in the course of surgery
- resection of necrotic bowel, formation of a double enterostomy
- after 4 weeks – enterostomy closure by placing T-shaped intestinal anastomosis (8 cases)
- enterostomy closure by placing end-to-end intestinal anastomosis (3 cases).

All surgical interventions were accompanied by adheziolysis due to the pronounced adhesive process between the intestinal loops and the peritoneum.

The T-shaped anastomosis is an end-to-side anastomosis when the end of the proximal lumen is anastomosed to the side of the distal intestinal segment directing the end of the distal intestinal segment out in the form of enterostoma to avoid from tension on anastomosis.

After the surgery, parenteral nutrition was prescribed with clinical and biochemical blood tests control. The duration of parenteral nutrition depended on the start of enteral nutrition and the change to physiological nutritional norm i. e. 10-14 days on average.

The mortality was 36.36% (4 children died). The main cause of death was severe sepsis with multiple organ failure in premature infants. Postoperative complication in the form of bowel stenosis in the area of the direct anastomosis was observed in 3 patients approximately 2 weeks after the surgery.

DISCUSSION

The results of our study showed that the severe condition of a newborn with NEC, with worsening clinical manifestations of the pathology, laboratory and instrumental studies and with symptoms of progressive intestinal obstruction against the background of adequate medicamentous therapy indicated the onset and development of obturative pathology. According to the three-stage classification proposed by M. Walsh and R. Kleigman [14], NEC with developing obturation intestinal obstruction refers to stage III of the disease, which is the stage of surgical implications development. The child's condition progressively worsened (increased intoxication, respiratory failure, edema syndrome) in the course of NEC treatment; the symptoms of intestinal obstruction aggravated within 2-3 days. 12 children were in serious condition, representing 46.15%. The condition of other 14 children (53.85%) was assessed as critical one.

Conservative treatment is carried out within 3 days with obligatory gastrointestinal decompression. Broad-spectrum antibiotics against aerobic and anaerobic bacteria are initially prescribed. After relevant microbiological studies, the course of antibiotic therapy is corrected. Nostimulatingmedicamentoustherapy.When gas and stool were returned to normal, conservative measures were continued until the child's condition was improved; and when there were no signs of stasis, minimal enteral feeding (1-2 ml of breast milk or formula per hour) was initiated. According to our information, con-

servative treatment was effective in 57.69% of newborns with obturative NEC.

In case of conservative treatment failure within 3 days, presence and/or worsening of clinical features of the intestinal obstruction, the surgery was indicated. Surgical treatment is initiated in patients with NEC which has clinical, roentgenologic, ultrasound signs of progressive intestinal obstruction against the background of conservative therapy failure.

The T-shaped anastomosis is an end-to-side anastomosis when the end of the proximal lumen is anastomosed to the side of the distal intestinal segment directing the end of the distal intestinal segment out in the form of enterostoma to avoid from tension on anastomosis.

The creation of a T-shaped anastomosis ensures:
- adequate emptying of the proximal intestinal segment
- the ability to introduce nutrient solutions into the distal intestinal segment, bypassing anastomosis
- wide junction between the proximal and distal intestinal segments
- the possibility to eliminate the stoma by excising the intestinal segment without intervention on anastomosis.

All practicing physicians need to know and remember about the possible occurrence and development of obturation intestinal obstruction in newborns with NEC. Timely treatment will make it possible to avoid fatal cases and the development of severe complications in a great number of patients.

CONCLUSIONS

1. NEC can lead to the development of obturation intestinal obstruction in newborns. Obturation intestinal obstruction was a form of NEC course in 18.18% of children under our observation.
2. Obturation intestinal obstruction in NEC in newborns is a reversible condition and requires conservative therapy. If symptoms of obstruction persist within 3 days after the start of conservative therapy, surgical treatment is indicated.
3. In case of obturation intestinal obstruction, it is advisable to perform resection of necrotic bowel, formation of a double enterostomy.
4. Enterostomy closure by placing T-shaped intestinal anastomosis should be performed 4 weeks after the primary surgery.
5. The use of modern techniques for early diagnosis and timely treatment of obturation intestinal obstruction in NEC can reduce mortality and the number of postoperative complications.
6. Newborns with NEC should be under the supervision of surgeons, which will help timely determine the indications for surgical intervention.

REFERENCES

1. Elgin T.G., Kern S.L., McElroy S.J. Development of the Neonatal Intestinal Microbiome and its association with Necrotizing Entarocolitis. Clin.Ther. 2016:38(4): 706 – 15.
2. Samuels N., van de Graaf R.A., de Jonge R.C.J., Reiss I.K.M. Risk factors for necrotizing enterocolitis in neonates: a systematic of prognostic studies. BMC Pediatr. 2017; 17(1): 105
3. Hackam D., Caplan M. Necrotizing enterocolitis: pathophysiology from a historical context. Seminars in Pediatr. Surgery. 2018; 27(1): 11 – 18.
4. Stol B.J., Hansen N.I., Bell E.F. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010; 126(3): 443 – 456.
5. Yee W.H., Soraisham A.S., Shah V.S. et al. Incidence and timing of presentation of necrotizing enterocolitis preterm infants. Pediatrics.2012; 129: 298 – 304.
6. Bajwa N.M., Berner M., Worley S., Pfister R.E. Swiss Neonatal Network. Population based age stratified morbidities of premature infants in Switzerland. Swiss Med Wkly. 2011; 141: 13212.
7. Yazji I. Endothelial TLR4 activation impairs intestinal microcirculatory perfusion in necrotizing enterocolitis via eNOS –NO-nitrite signaling. Proc. Natl Acad Sci USA. 2013; 110: 9451 – 56.
8. Neu J., Pammi M. Pathogenesis of NEC: impact of an altered intestinal microbiome. Semin Perinatol. 2017; 41: 29 – 35.
9. Denning N.L., Prince J.M. Neonatal intestinal dysbiosis in necrotizing enterocolitis. Molecular Medicine. 2018; 24: 4.
10. Sharma R., Hudak M.L. A clinical perspective of Necrotizing Enterocolitis: past, present and future. ClinPerinatol. 2013; 40(1): 27 – 51.
11. Neu J., Pammi M. Pathogenesis of NEC: impact of an altered intestinal microbiome. Semin Perinatol. 2017; 41: 29 – 35.
12. Gephart S.M., Fleiner M., Kijewski A. The connection between abdominal signs and necrotizing enterocolitis in infants 501 to 1500g. Adv. Neonatal Care. 2017; 17(1): 53 – 64.
13. Estaueb A.A., Mostafa M.M., Ibrahim N.H. The role of surgery in management of necrotizing enterocolitis. Int. J. Surg. 2010; 8(6): 458 – 461.
14. Walsh M., Kleigman R. Necrotizing enterocolitis: treatment based on staging criteria. Ped. Am. 1986; 33: 179 – 201.
15. Bairov V.G., Amidkhonova S.A., Shchegoleva N.A. et al. Criteria for the choice of a method for the construction of anastomosis in newborns with small bowel obstruction. Ped.Surg. 2015; 19(1): 15 – 20.

ORCID and contributionship:

Olga M. Gorbatyuk: 0000-0003-3970-8797 [A,B,E]
Stanislav I. Babuci: 0000-0002-1153-4871 [A,B,F]
Taras V. Martinyuk: 0000-0002-0488-9148 [B,C,F]
Konstantin T. Bertsun: 0000-0002-2612-3709 [B,C,D]

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olga M. Gorbatyuk
Shupyk National Medical Academy of Postgraduate Education
9 Dorogozhitska str. 04112 Kyiv, Ukraine
tel: +380503820641
e-mail:ol.gorbatyuk@gmail.com

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ORIGINAL ARTICLE

DIGITAL BREAST TOMOSYNTHESIS AND FULL-FIELD DIGITAL MAMMOGRAPHY IN BREAST CANCER DETECTION ASSOCIATED WITH FOUR ASYMMETRY TYPES

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Andrii V. Gurando[1,4], Tetiana M. Babkina[2], Iryna M. Dykan[1], Tetiana M. Kozarenko[2], Viacheslav R. Gurando[3], Vadim V. Telniy[4]
[1]SI, "INSTITUTE OF NUCLEAR MEDICINE AND DIAGNOSTIC RADIOLOGY OF NATIONAL ACADEMY OF MEDICAL SCIENCE OF UKRAINE", KYIV, UKRAINE
[2]P.L. SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KYIV, UKRAINE
[3]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[4]MEDICAL CENTER, «VERUM EXPERT», KYIV, UKRAINE

ABSTRACT

The aim: Comparing sensitivity and specificity of digital breast tomosynthesis and full-field digital mammography in breast cancer detection associated with four different types of asymmetries according to BI-RADS Atlas.

Materials and methods: Study included 201 patients with four types of asymmetries according BI-RADS atlas (asymmetry – 81 (40,3%), focal asymmetry – 82 (40,8%), global asymmetry – 36 (17,9%) and developing asymmetry – 2 (1,0%)) who underwent full-field digital mammography, digital breast tomosynthesis and hand-held full breast ultrasound from January 2017 to June 2018.

The general rate of breast cancer for the 201 patients with asymmetries was 8 cases (4,0%) (IBC, n=6 (3,0%); DCIS, n=2 (1,0%) other findings associated with asymmetries were non-malignant, n=10 (5,0%) (sclerosing adenosis, n=5 (2,5%); fibroadenomatosis, n=3 (1,5%); simple cyst, n=1 (0,5%); radial scar associated with papilloma, typical ductal hyperplasia and sclerosing adenosis, n=1 (0,5%).

Results: Analysis of the results showed that sensitivity of digital breast tomosynthesis was 75.0% [95% CI, 34.91% to 96.81%] and specificity was 94.8% [95% CI, 90.68% to 97.49%] which was superior to full-field digital mammography sensitivity 50.0% [95% CI, 15.70% to 84.30%] and specificity 91.19% [95% CI, 86.27% to 94.78%] for breast cancer detection associated with different types of asymmetries.

Conclusions: Using of digital breast tomosynthesis in assessment of breast asymmetries can improve sensitivity and specificity in breast cancer detection and reduce number of unnecessary biopsies and short-interval follow-up examinations.

KEY WORDS: Asymmetries, digital breast tomosynthesis, mammography

Wiad Lek. 2021;74(4):842-848

INTRODUCTION

According to the current data of national cancer-register breast cancer (BC) takes the first place among oncologic diseases in Ukrainian woman [1].

Large studies showed that screening mammography can reduce BC mortality in population approximately for 40% [2-4]. But dense fibroglandular breast tissue, due to its overlapping effect, still reduces sensitivity of full-field digital mammography (FFDM) to 48.0% – 62.9% in dense breasts in comparison with high FFDM sensitivity for fatty breasts 85.7%–88.8% [5-7].

Corresponding to the last investigations – digital breast tomosynthesis (DBT) can improve BC detection in women with dense breasts and reduce recall rate, especially for asymmetries, due to 3D visualization of breast patterns [8-12].

Although there is clearly wide variation in breast size and parenchymal pattern, the breasts are generally symmetric structures with similar density and architecture [13]. However, asymmetries account for 6% of non–palpable, screen–detected cancers, represent 27% of missed cancers and are the second most common cause of false negative screening recall assessments [14-17].

The term asymmetric breast tissue refers to a greater volume or density of breast tissue in one breast than in the corresponding area in the contralateral breast, in contrast to the mass, which is three-dimensional, asymmetric findings is often normal fibroglandular tissue with interspersed fat and lacking convex margins, which is caused by the superimposition artifact in a given mammographic projection, but, it can be possible that the asymmetry, in fact, is a cancer [13,14,18].

The last 5-th edition of Breast Imaging – Report and Data System Atlas (BI-RADS) divided asymmetric breast densities into four categories: asymmetry, focal asymmetry, global asymmetry and developing asymmetry [18].

Detection and diagnostic evaluation of asymmetries is particularly challenging, as many appear similar to

Table I. Patients with four types of asymmetries assessed, by using BI-RADS categories, separately for FFDM and for DBT.

Asymmetry type	BI-RADS categories for FFDM					BI-RADS categories for DBT				
	1	2	3	4	5	1	2	3	4	5
One-view asymmetry	0	23	50	8	0	12	55	10	4	0
Focal asymmetry	0	17	53	12	0	0	52	19	7	3
Global asymmetry	0	36	0	0	0	0	36	0	0	0
Developing asymmetry	0	0	0	2	0	0	0	0	2	0

Table II. ACR breast density distribution for each asymmetry type.

Asymmetry type	ACR density types				
	a	b	c	d	total
One-view asymmetry	0	28	51	2	81
Focal asymmetry	0	37	45	0	82
Global asymmetry	0	5	30	1	36
Developing asymmetry	0	0	2	0	2
total	0	70	128	3	201

Table III. Breast cancer involvement depending on the type of asymmetry.

Asymmetry type	Number of cases	Biopsy performed	Ca detected
One-view asymmetry	81	4	1
Focal asymmetry	82	12	6
Global asymmetry	36	0	0
Developing asymmetry	2	2	1

fibroglandular tissue at mammography [19]. At mammographic examinations they have low but clinically important yield of invasive cancer and are common source of false-positive results, particularly among women younger than 70 years, but that not all cancers present with the 'typical' features of malignancy [14, 20]. Asymmetry which cannot be attributed to a benign cause should undergo biopsy to avoid a delayed diagnosis of breast cancer [14].

THE AIM

The aim of our study was to compare sensitivity and specificity of DBT and FFDM in BC detection associated with different types of asymmetries.

MATERIALS AND METHODS

Study included 201 patients with four types of asymmetries according to BI-RADS atlas (asymmetry – 81 (40,3%), focal asymmetry – 82 (40,8%), global asymmetry – 36 (17,9%) and developing asymmetry – 2 (1,0%)) who underwent FFDM, DBT and hand-held full breast *ultrasound* (HHUS) from January 2017 to June 2018. All asymmetries were assessed by using BI-RADS categories separately for FFDM and for DBT [Table I].

Suspicious asymmetries underwent morphological verification, n=18 (9,0%), probably benign and benign asymmetries were followed up for 24-30 months after the first mammographic study to ensure long-term stability of what is almost certainly benign pathology, n=183 (91%). During this time none of the patients underwent biopsy on the basis of one-view asymmetry and global asymmetry, while 2 developing asymmetries, which were focal asymmetries at the first examination, underwent CNB and one of it revealed DCIS.

Exclusion criteria for our study were: absence of asymmetries, lack of morphological verification for suspicious findings or absence of 2 years follow up for benign and probably benign findings, previous history of surgery or trauma concordant with asymmetry.

Bilateral FFDM and DBT were performed in two standard projections (CC and MLO) on Selenia Dimensions Mammography system (Hologic, USA). Stereotactic biopsies were performed on Hologic MultiCare Platinum Prone Breast Biopsy Table, when there wasn't possibility to establish correlation with HHUS and perform ultrasound-guided core needle biopsy (CNB).

HHUS and ultrasound-guided biopsies were performed on a Toshiba Viamo and Toshiba Aplio XG ultrasound systems with a linear probe centered at 9,0MHz.

Age of the patients ranged from 37 years to 70 years with mean age of 45,4 years. Patient with suspicious asymmetries underwent 16 ultrasound-guided and 2 stereotactic CNB, and revealed 8 malignant and 10 benign lesions.

Retrospectively, all studies with previously FFDM detected asymmetries, in consensus, were read by two dedicated breast radiologist. First only FFDM images in two standard projections (CC, MLO) were read and assessed by using

BI-RADS categories, without knowing results of DBT examination and pathology, and then DBT images were assessed by using BI-RADS categories.

RESULTS

The overall rate of BC involvement for the 201 patients with asymmetries was 8 cases (4,0%) (invasive breast cancers (IBC), n=6 (3,0%); ductal cancer in situ (DCIS), n=2 (1,0%) other findings associated with asymmetries were non-malignant, n=10 (5,0%) (sclerosing adenosis, n=5 (2,5%); fibroadenomatosis, n=3 (1,5%); simple cyst, n=1 (0,5%); radial scar associated with papilloma, typical ductal hyperplasia and sclerosing adenosis, n=1 (0,5%) [Table III].

Nine percent (18/201) of the lesions were biopsied. We performed 16 ultrasound-guided CNB, while the remainder, when we couldn't find correlation between mammographic and sonographic picture, underwent stereotactic CNB (2/18) .

All (n=36) global asymmetries were assessed identically by both methods, FFDM and DBT, BI-RADS-2, after ultrasound and 2 years of follow-up asymmetric fibroglandular tissue didn't change.

Among one-view asymmetries (n=81) we performed 4 biopsies and detected one BC.

Case Report №1. One-view asymmetry representing malignancy. (Fig.1) CC and (Fig.2) MLO views: a potential lesion lacking the characteristics of a mass is seen only on the (Fig.1) LCC view (red square), a corresponding abnormality is not seen on the (Fig.2) LMLO view. (Fig.3) RCC tomo scan view and US scan showed an irregular hypoechoic mass with indistinct margins and vascularity (red square) assessed as BI-RADS category 4. Pathologic diagnosis revealed ductal cancer in situ.

Due to DBT we avoided 4 unnecessary biopsies of benign breast findings which were stable during next 2 years, by lowering BI-RADS-4 category to BI-RADS-3 category. Also we avoided short-time follow-up examinations in 40 cases by lowering BI-RADS-3 category to BI-RADS-2 or even BI-RADS-1 category.

Case Report №2. One-view asymmetry representing summation artifact. (Fig.4) CC and (Fig.5) MLO views: asymmetry is seen only on the RCC view (red square). (Fig.6) On the RCC tomo scan view, the asymmetry didn't disappear. (Fig.6) Echo-scan, obtained for further evaluation, demonstrates an island of normal breast tissue that corresponds to the one-view asymmetry seen at FFDM. Follow-up mammography after 24 months showed no interval change.

We found 6 BC and made 12 biopsies among 82 focal asymmetries.

Case Report №3. Focal asymmetry representing malignancy. (Fig.7) CC and (Fig.8) MLO views show a focal asymmetry in the retroareolar region of the right breast (red square). (Fig.7-8) RCC and RMLO tomo scan views detected an irregular mass with indistinct margins that was superimposed by fibroglandular breast tissue in the retroareolar region of the right breast. Pathology confirmed invasive carcinoma of no special type.

Three cases of BC represented as focal asymmetry were underestimated on FFDM and assessed as BI-RADS-3 finding. Five cases were overestimated by using BI-RADS-4 category and lowers to BI-RADS-3 category by using DBT. BI-RADS-3 category was used only in 19 cases after DBT examination instead of 53 cases during FFDM examination.

Case Report №4. Focal asymmetry representing summation shadow. (Fig.9) CC and (Fig.10) MLO views show a focal asymmetry in the right upper outer quadrant (red square). On the (Fig.9-10) RCC and RMLO tomo scans asymmetry lost its density and ultrasound (not shown) did not demonstrate any abnormal findings in the corresponding region. Follow-up mammography after 26 month showed no interval change.

We had only two cases of developing asymmetries which were focal asymmetries on initial FFDM and DBT examinations. In both cases, after negative dynamic were seen, findings were assessed as BI-RADS-4 lesions. One of these finding was ductal cancer in situ and other lesion was radial scar associated with papilloma, typical ductal hyperplasia and sclerosing adenosis.

The difference in assessment for BI-RADS-2 and 3 categories for all types of asymmetries by using FFDM and DBT was statistically significant ([95% CI, 12.27–22.08]; p=0.00001).

Analysis of the results showed that sensitivity of DBT was 75.0% [95% CI, 34.91% to 96.81%] and specificity was 94.8% [95% CI, 90.68% to 97.49%] which was superior to FFDM sensitivity 50.0% [95% CI, 15.70% to 84.30%] and specificity 91.19% [95% CI, 86.27% to 94.78%] for BC detection associated with different types of asymmetries.

DISCUSSION

DBT is a relatively new modality for Ukrainian breast imaging. First experience of 3D mammography in Ukraine showed superior sensitivity for BC detection by using DBT than FFDM [21].

Another study showed that adding of DBT at screening mammography has lowered the number of callbacks of findings due to summation artifact and elevated the diagnostic accuracy of radiologists for BC detection [19].

In line with other studies, after FFDM and DBT we evaluated all asymmetries with targeted diagnostic HHUS and, in some cases, we performed spot-compression views on region of interest [19, 22, 26,]. When we saw at ultrasound typical fibroglandular breast tissue which correlates with asymmetric breast finding, then patient was returned to routine follow-up mammography examinations. But when suspicious lesion was found in the area of interest, than CNB with US-guidance was performed. However, in 2 cases mammographically suspicious asymmetries were verified with stereotactic guidance, when we couldn't surely find correlation between asymmetric mammographic finding and ultrasound images in the region of interest.

According to the literature, asymmetry (area of fibroglandular-density tissue that is visible on only one mam-

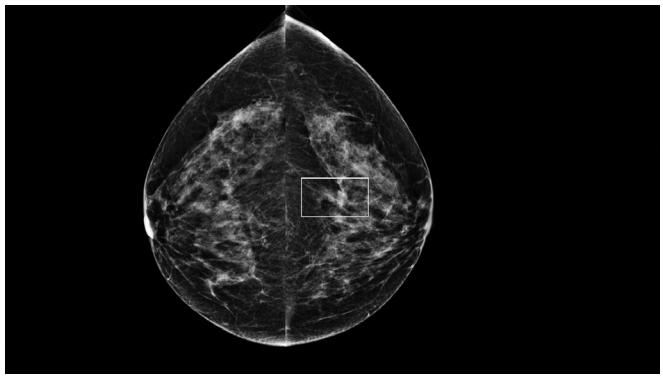

Fig. 1. RCC and LCC views

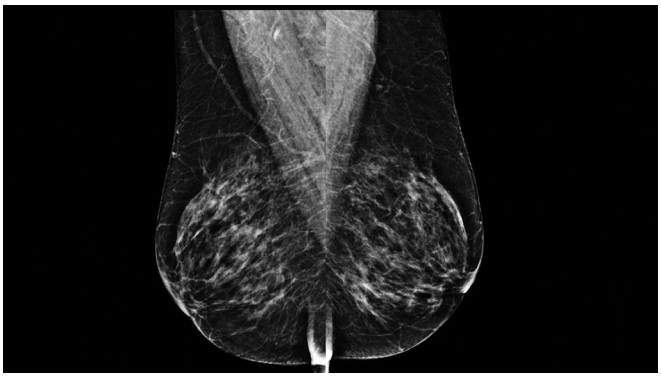

Fig. 2. RMLO and LMLO views

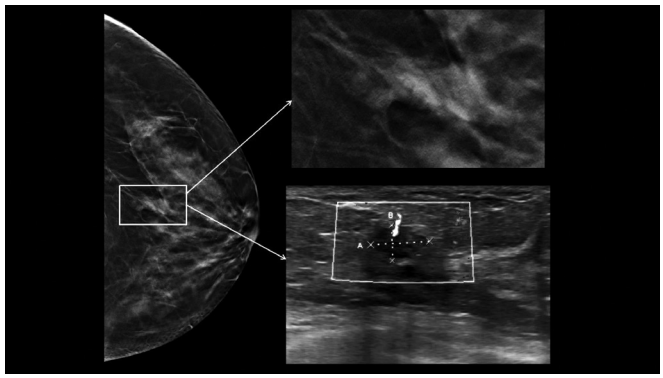

Fig. 3. Correlation between DBT images and US scan of suspicious lesion

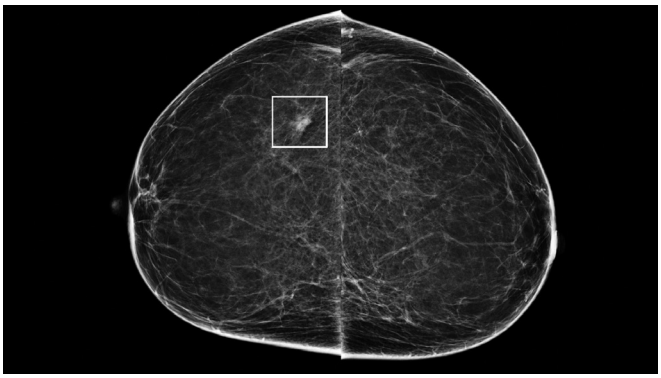

Fig. 4. RCC and LCC views – asymmetry is seen only on the right CC view (red square)

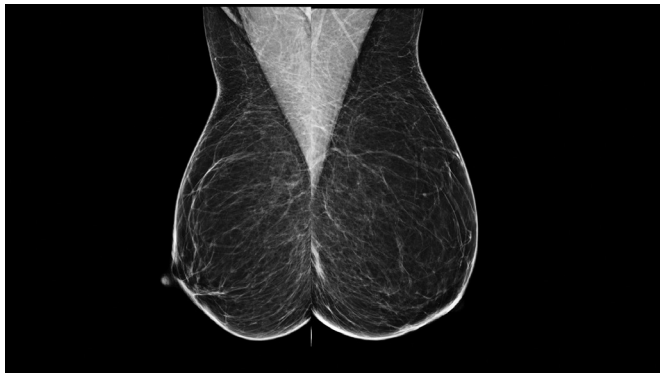

Fig. 5. RMLO and LMLO views

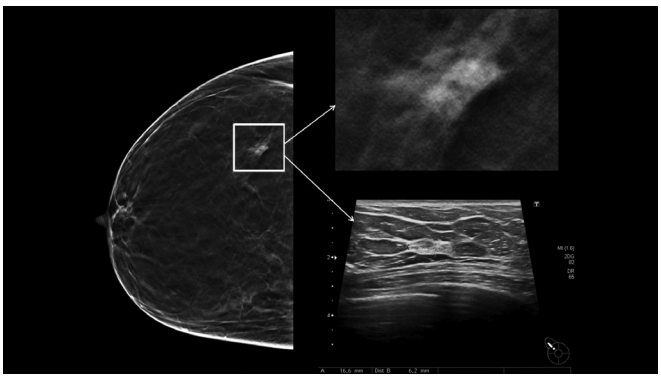

Fig. 6. Correlation between DBT images and US scan which demonstrates an island of normal breast tissue that corresponds to the asymmetry

mographic projection) occurs 3,3%-20,5% of screening mammograms and its likelihood of malignancy is 1,8%-3,6%, but radiologists often recall patients with abnormal one-view-only findings for additional imaging, because it can be cancer which is obscured by dense tissue on the other view or can be outside of image field [20]. Most of one-view-only findings represent fibroglandular tissue without any changes, and represents cancer very rare. Nevertheless, some asymmetries after additional examinations can be reclassified as mass or architectural distortion and increase breast cancer detection, in our study among 81 cases of one-view asymmetries we performed 4 biopsies and detected 1 invasive breast cancer [18-23].

Focal asymmetry – is judged relative to the corresponding location in the contralateral breast, and represents a relatively small amount of fibroglandular-density tissue over a confined portion of the breast (less than one quadrant) – occurs 0.87% – 21.4% of screening mammograms, overall likelihood of malignancy of 0.67% – 3.7%, in our study among 82 cases of focal asymmetries we performed 12 biopsies and detected 6 cancers [18-20, 24].

Global asymmetry – is judged relative to the corresponding area in the contralateral breast and represents a large amount of fibroglandular-density tissue over a substantial portion of the breast (at least one quadrant) and occurs about 3.0% of mammograms and should be considered

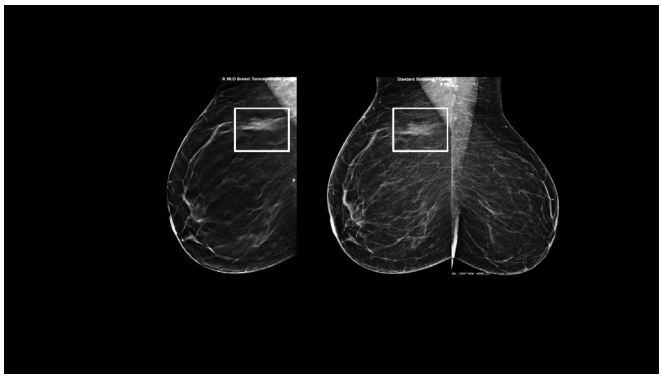

Fig. 7. RCC FFDM, DBT and LCC views – asymmetry is seen on the RCC views (red square)

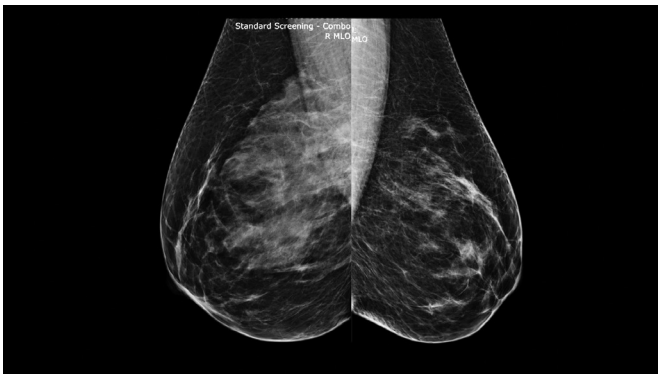

Fig. 8. RMLO FFDM, DBT and LMLO views – asymmetry is seen on the RMLO views (red square)

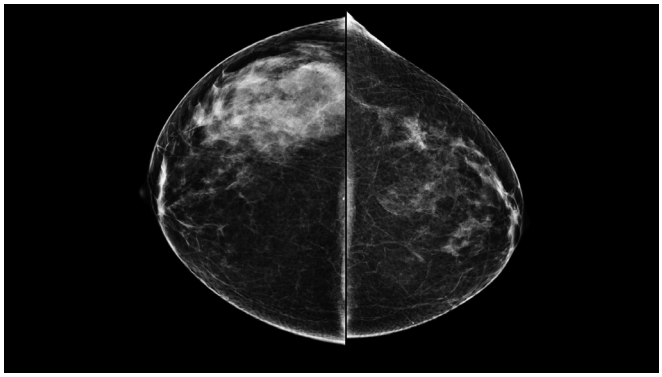

Fig. 9. RCC FFDM, DBT and LCC views – asymmetry is seen on the RCC views (red square)

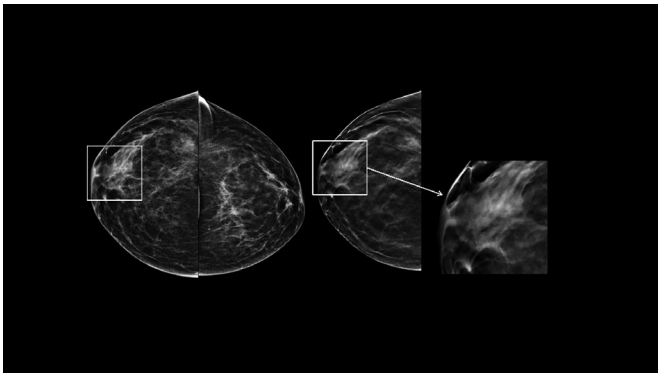

Fig. 10. RMLO FFDM, DBT and LMLO views – asymmetry is seen on the RMLO views (red square)

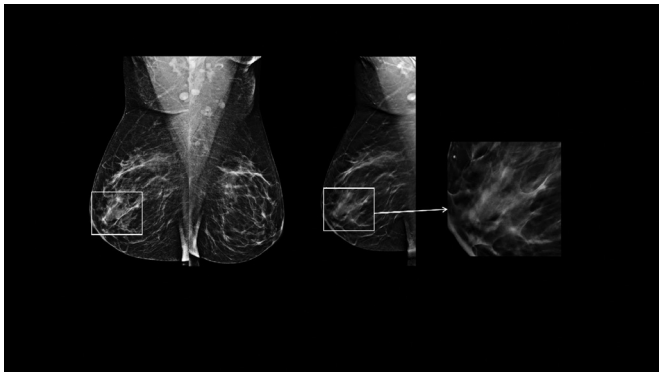

Fig. 11. Global asymmetry seen on RMLO view in upper outer quadrant of the right breast (red oval)

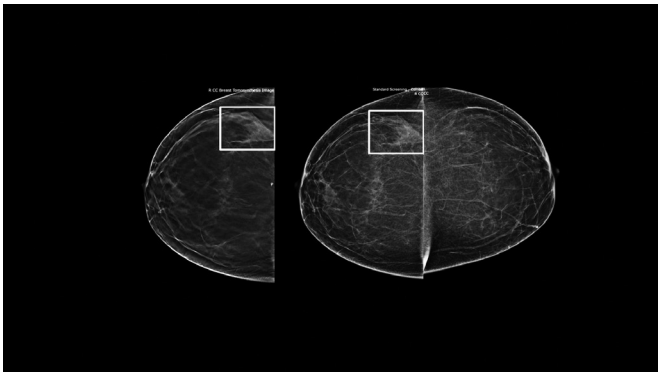

Fig. 12. Global asymmetry seen on RCC view in upper outer quadrant of the right breast (red oval)

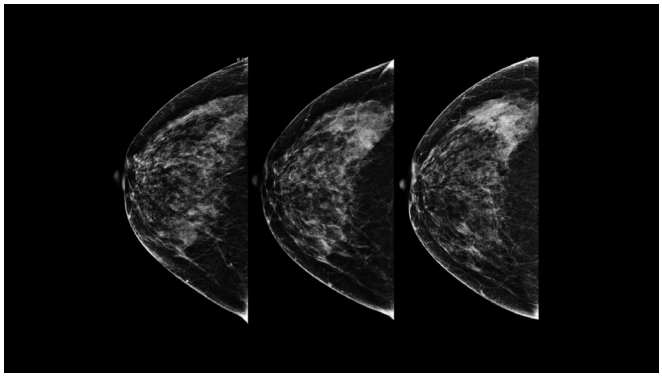

Fig. 13. RCC views show that in lateral quadrants (red oval) has developed over 2 years, larger and larger and more conspicuous asymmetry.

as a normal variant [18, 20, 25, 26]. In our study among 36 cases of global asymmetry no biopsies were performed and no breast cancers were detected during 24-28 months of follow-up after initial mammographic study.

Case Report №5. Global asymmetry seen on (Fig. 11) MLO and (Fig. 12) CC mammographic views in right upper outer quadrant of the right breast (red oval) represents a much greater volume of breast tissue relative to the corresponding region in the left breast with no associated mass, suspicious calcifications, or architectural distortion. Follow-up mammography in 12 and then 25 months from initial examination showed no interval change.

Developing asymmetry – is a focal asymmetry that is new, larger, or more conspicuous than on a previous ex-

amination and occurs 0,11%-4,4% of all mammograms, predictive of cancer seen on 7,4% to 26,7% [15, 18, 19, 20]. This type of asymmetries can have benign (cyst, fibrocystic change, pseudoangiomatous stromal hyperplasia, scar, focal infections, weight loss or gain, trauma, fat necrosis, and hormone replacement therapy) and malignant causes invasive ductal and lobular cancers, invasive mucinous carcinoma and DCIS [19]. In our study we had 2 developing asymmetries, in both cases we performed CNB and revealed in first case DCIS and in second case radial scar associated with papilloma, typical ductal hyperplasia and sclerosing adenosis. Also case of DCIS associated with developing asymmetry was classified as false-negative due to diagnostic character of mammography.

Case Report №6. Developing asymmetry representing malignancy. (Fig. 13) RCC mammographic views show that in lateral quadrants (red oval) has developed over 2 years, larger and more conspicuous asymmetry than on previous examinations, now called a developing asymmetry. It was found to be ductal cancer in situ.

CONCLUSIONS

The results of our study suggest that DBT can better differentiate benign breast asymmetries, which are due to summation artifact, from breast cancers which may appear similar to normal fibroglandular tissue at FFDM. Depending on asymmetric finding, defined by BI-RADS atlas, we can presuppose the likelihood of cancer after mammographic examination.

In this study, developing asymmetry showed the highest rate of BC involvement (1/2), on the second place was focal (6/82) and on the third place was one-view asymmetry (1/81), among global asymmetries there were no breast cancers detected during two years of follow-up. Using of DBT in assessment of breast asymmetries can improve sensitivity and specificity in breast cancer detection and reduce number of unnecessary biopsies and short-interval follow-up examinations.

REFERENCES

1. Fedorenko Z.P., Michailovich Y.Y., Goulak L.O. et al. Cancer in Ukraine 2018 to 2019, incidence, mortality, activities of oncological service. In: Kyiv: National Cancer Institute of Ukraine. 2020: 42-43.
2. Broeders M., Moss S., Nyström L. et al. The impact of mammographic screening on breast cancer mortality in Europe: a review of observational studies. J Med Screen. 2012;19(1):14-25. doi:10.1258/jms.2012.012078.
3. Weedon-Fekjær H., Romundstad P.R., Vatten L. J. Modern mammography screening and breast cancer mortality: population study. BMJ. 2014; 348:3701.
4. Nyström L., Andersson I., Bjurstam N. et al. Long-term effects of mammography screening: updated overview of the Swedish randomised trials. The Lancet. 2002. doi.org/10.1016/S0140-6736(02)08020-0.
5. Carney P.A., Miglioretti D.L., Yankaskas B.C. et al. Individual and combined effects of age, breast density, and hormone replacement therapy use on the accuracy of screening mammography. Ann Intern Med. 2003; 138:168–175. doi:10.7326/0003-4819-138-3-200302040-00008.
6. Kolb T.M., Lichy J., Newhouse J.H. Comparison of the performance of screening mammography, physical examination, and breast US and evaluation of factors that influence them: an analysis of 27,825 patient evaluations. Radiology. 2002; 225:165–175. doi: 10.1148/radiol.2251011667.
7. Kerlikowske K., Hubbard R.A., Miglioretti D.L. et al. Comparative effectiveness of digital versus film-screen mammography in community practice in the United States: a cohort study. Ann Intern Med. 2011;155(8):493–502.
8. Durand M.A., Haas B.M., Yao X. et al. Early clinical experience with digital breast tomosynthesis for screening mammography. Radiology. 2015; 274:85–92. doi: 10.1148/radiol.14131319.
9. Lourenco A.P., Barry-Brooks M., Baird G.L. et al. Changes in recall type and patient treatment following implementation of screening digital breast tomosynthesis. Radiology. 2015; 274:337–342. doi: 10.1148/radiol.14140317.
10. Rose S.L., Tidwell A.L., Bujnoch L.J. et al. Implementation of breast tomosynthesis in a routine screening practice: an observational study. AJR Am J Roentgenol. 2013; 200:1401–1408. doi: 10.2214/AJR.12.9672.
11. Babkina T.M., Dykan I.M., Gurando A.V. et al. Detection of breast cancer presenting as a mass in women with dense breasts - digital breast tomosynthesis versus full-field digital mammography. Exp Oncol. 2020;42(3):215-219. doi:10.32471/exp-oncology.2312-8852.vol-42-no-3.14898.
12. Dykan I.M., Bozhok Ye.M., Gurando A.V. et al. Cyfrovyi tomosyntez v diagnostytsi zahvoryuvan grudnyh zaloz; rozkish chy neobhidnist?(Analytychnyi oglyad literature) [Digital tomosynthesis in the diagnosis of breast diseases: luxury or necessity? (Analytical review of the literature)].Woman's Health.2017; 8(124):108-115. (in Ukrainian).
13. Samardar P., de Paredes E.S., Grimes M.M. et al. Focal Asymmetric Densities Seen at Mammography: US and Pathologic Correlation. 2002. doi.org/10.1148/radiographics.22.1. g02ja2219.
14. Peart J., Thomson G., Wood S. Developing asymmetry in a screening mammogram: A cautionary tale of a missed cancer. J Med Imaging Radiat Oncol. 2018;62(1):77-80. doi: 10.1111/1754-9485.12677.
15. Leung J.W., Sickles E.A. Developing asymmetry identified on mammography: correlation with imaging outcome and pathologic findings. AJR. 2007; 188: 667–75.
16. Hoff S.R., Abrahamsen A.L., Samset J.H. et al. Breast cancer: missed interval and screening–detected cancer at full–field digital mammography and screen–film mammography– results from a retrospective review. [Erratum appears in Radiology. 2013 Jan; 266(1):367]. Radiology 2012; 264: 378–86.
17. Ciatto S., Houssami N., Ambrogetti D. et al. Minority report – false negative breast assessment in women recalled for suspicious screening mammography: imaging and pathological features, and associated delay in diagnosis. Breast Cancer Res Treat. 2007; 105:37–43.
18. Sickles E.A., D'Orsi C.J., Bassett L.W. et al. ACR BI-RADS mammography. In: ACR BI-RADS atlas Breast Imaging Reporting and Data System 2013. 5th ed. Reston, Va: American College of Radiology. 2013;1–175.
19. Chesebro A.L., Winkler N.S., Birdwell R.L. et al. Developing Asymmetries at Mammography: A Multimodality Approach to Assessment and Management. RadioGraphics.2010; 36:2. doi.org/10.1148/rg.2016150123.
20. Venkatesan A., Chu P., Kerlikowske K. et al. Positive predictive value of specific mammographic findings according to reader and patient variables. Radiology. 2009;250(3):648-657. doi:10.1148/radiol.2503080541.

21. Dykan I.M., Bozhok Ye.M., Gurando A.V. Pershyi dosvid vykorystannia 3D mamohrafii v Ukraini. [The first experience of using 3D mammography in Ukraine]. Luchevaja diagnostika. Luchevaja Terapija. 2018; 2:40-8. (in Ukrainian).
22. Bozhok Ye.M., Gurando A.V. Imitatsiya raku lokalnoyu asymetriyeyu grudnoi zalozy. Klinichny vypadok. [Imitation of cancer by local asymmetry of the breast. Clinical case.]. Luchevaja diagnostika. Luchevaja Terapija 2017; 2:68-73. (in Ukrainian).
23. Sickles E.A. Findings at mammographic screening on only one standard projection: outcomes analysis. Radiology. 1998;208(2):471–475.
24. Sickles E.A. The spectrum of breast asymmetries: imaging features, work-up, management. Radiol Clin North Am. 2007;45(5):765–771.
25. Kopans D.B., Swann C.A., White G. et al. Asymmetric breast tissue. Radiology. 1989;171(3):639–643.
26. Youk J.H., Kim E.K., Ko K.H. et al. Asymmetric Mammographic Findings Based on the Fourth Edition of BI-RADS: Types, Evaluation, and Management. RadioGraphics. 2009; 29(1). doi.org/10.1148/rg. e33.

ORCID and contributionship:
Andrii V. Gurando: 0000-0002-2708-3040 [B,D]
Tetiana M. Babkina: 0000-0001-5046-870X [A]
Iryna M. Dykan: 0000-0001-8776-3278 [E]
Tetiana M. Kozarenko: 0000-0002-0838-9773 [E]
Viacheslav R. Gurando: 0000-0001-6303-3799 [F]
Vadim V. Telniy: 0000-0001-9860-9663 [C]

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CORRESPONDING AUTHOR
Andrii V. Gurando
SI "Institute of Nuclear medicine and diagnostic Radiology of National Academy of Medical Science of Ukraine"
32 Platona Maiborody St., 04050 Kyiv, Ukraine,
tel:+38(096) 273-92-73
e-mail: avgour@gmail.com

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ORIGINAL ARTICLE

DYNAMIC OF UPPER LIMB SENSORIMOTOR RECOVERY ASSESSED ON THE FUGL-MEYER SCALE IN POST-STROKE PATIENTS WITH NEGLECT SYNDROME RECEIVING COMBINED PHYSICAL THERAPY AND ERGOTHERAPY

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Yurii M. Dido, Olena A. Dulo
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To assess the effect of the modified combined program of physical therapy and ergotherapy on the indicators of upper limb recovery in the patients with the left unilateral neglect.

Materials and methods: The study involved 58 patients diagnosed with the right hemisphere stroke followed by neglect syndrome. Patients were randomly divided into main group (MG) and control group (CG) according to the ratio of 1:1. Physical therapy of CG patients included proprioceptive neuromuscular facilitation (PNF), balance training, ergotherapeutic intervention, and exercises improving fine motor skills. Specially developed intervention program of MG patients took into account patient's individual capabilities and needs. It included PNF, balance training and upright posture correction, constraint-induced movement therapy, dual task activities, stimulation of the affected side in daily activities, targeted therapy. The length of intervention comprised 3 months in both groups.

Results: According to the results of the primary examination, the groups had no differences in demographic variables, clinical history (NIHSS scale, the Glasgow Coma Scale and Albert's Test) and Fugl-Meyer assessment of upper limb sensorimotor recovery. Statistical analysis of the final scores of the Fugl-Meyer scale confirmed that MG had statistical advantages in all measured items of the motor function domain, as well as in a number of proprioceptive sensitivity indicators.

Conclusions: The obtained results confirm better efficacy of the modified program of physical therapy and ergotherapy, received by MG patients.

KEY WORDS: left unilateral neglect, balance training, constraint-induced movement therapy, dual task activities

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INTRODUCTION

The consequences of past cerebral circulatory disorders lead to a serious decrease of patients' life quality. Being the main reason for progressive and long-term disability of the population, stroke remains one of the most important challenges of modern society [1,2].

Depending on the location and extent of brain morphological changes following the stroke, the clinical picture and movement disorders can vary significantly [3,4].

A few days after onset, when cerebral disorders are smoothed, priority is taken by movement disorders, which depend on the location of the pathological process. Movement disorders in the acute period develop in 75% of patients, and six months later movement defects persist in 53% of patients [3].

Neglect syndrome is observed in case of large damages of back (parietal or parieto-occipital) lobes mainly of the right hemisphere. There are opinions that the development of this syndrome involves thalamus, basal ganglia, corpus callosum, frontal, parietal and temporal lobes, reticular formation, other non-specific parts of the brain affected by stroke [5-7].

According to various authors, this syndrome is observed in 33-85% of patients with right hemispheric stroke and 24% of patients with left hemispheric stroke [8].

Such patients do not tell about their complaints in the conversation, considering themselves "completely healthy" and claiming that their left limbs function just as well as the right limbs. Patients are passive about the existence of the disorder, rehabilitation process and obtained positive results, and usually cannot sufficiently implement their movement functions [9, 10].

Spatial (visual) neglect can be diagnosed with the help of Albert's test, which enables to detect and quantify it. The percentage of missed segments correlates with the degree of cognitive function restoration six months after the test [1].

On the other hand, one of the most important causes of disability after stroke is arm impairment, which occurs in 70% of the patients with past acute cerebral circulatory disorders [11]. In this case upper limb function recovery usually [12] occurs at a later date, often remaining the only reason for patient's disability. Upper limb function is completely restored only in 20% of patients [11,13,14].

The main approach, continually applied in the treatment and rehabilitation of neglect, directly addresses the problem of the main deficit of neglect and attempts to refocus attention on the neglected side. Visual scanning therapy is widely used in neglect rehabilitation, basically encouraging

the patients to study, for example, the left side of space, often with the help of visual signals [15,16].

Neglect following acute cerebral circulatory disorder was proved to affect the quality of rehabilitation [15], namely to prolong the length of hospital stay, increase the risk of falling, reduce the chances of returning home after intensive inpatient rehabilitation, i.e. the patient with neglect cannot be completely self-supporting [17].

Taking into account the available data, improving the results of physical therapy and ergotherapy in stroke patients with neglect syndrome is an important task.

THE AIM

The aim was to assess the effect of the modified combined program of physical therapy and ergotherapy on the indicators of upper limb sensorimotor recovery in the patients with the right hemisphere ischemic acute cerebral circulatory disorders followed by neglect syndrome.

MATERIALS AND METHODS

Participants: The study involved 58 patients (29 females and 29 males) diagnosed with the right hemisphere ischemic acute cerebral circulatory disorder followed by neglect syndrome. All persons were informed about the content of the tests, measurement procedures and signed an informed consent form. The research was approved by the Institutional Ethics Committee (number 1/2017) and was carried out in compliance with the international principles of the Helsinki Declaration of the World Medical Association [18], and in accordance with the Law of Ukraine "Fundamentals of Ukrainian Legislation on Healthcare" [19] on ethical norms and rules for conducting medical research involving human.

The patients were randomly divided into main group (MG) and control group (CG) according to the ratio of 1:1. The exclusion criteria were hemianopsia, scores above 3 on the Scale of Contraversive pushing, scores above 14 on the NIHSS scale.

Setting: hospitalized care and home-based rehabilitation program.

Interventions: Physical therapy of CG patients included proprioceptive neuromuscular facilitation (PNF), balance training, ergotherapeutic intervention, and exercises impoving fine motor skills. Specially developed intervention program of MG patients took into account patient's individual capabilities and needs, after analyzing the obtained results. The Predict Recovery Potential (PREP2) algorithm was used to predict upper limb recovery interventions. Goal setting employed SMART goals framework. The SOAP format was used for therapy planning.

PNF (scapular and upper limb patterns), constraint-induced motor therapy (SIMT) and dual task activities were used. The intervention included balance exercises while sitting and standing, as well as neglect correcting exercises, namely:
- stimulation of the affected side (tactile, visual, motor);
- constant drawing attention to the affected side of the space (wearing bright bracelets on the left hand, placing photos of significant people and bright objects on the left side);
- involvement of the affected side in daily activities (placing food (not hot) on the affected side, approaching the patient by specialists and relatives from the neglected side, placing board games, means of communication on the neglected side);
- patients were also asked to place clock hands according to the given time; complete missing parts of the objects, letters and numbers in the pictures; replicate specific patterns in the Kohs Block test, create patterns using tactile (tactile-colored) dominoes; find the middle of the length; cross out the numbers in the right and left halves of the sheet; work on sheets divided in half; draw symmetrical pictures; move hands alternately and simultaneously, etc.

The therapy was performed 5 days a week for 14±4.2 days, and then 3 times a week for up to three months in both groups.

Three months later, the patients had a repeated, final examination.

Outcome Measures: Demographic variables, clinical history (NIHSS scale, the Glasgow Coma Scale and Albert's Test scores). Comparing the efficacy of the combined program of physical therapy and ergotherapy in CG and MG was carried out using indicators of upper limb sensorimotor recovery according to Fugl-Meyer scale.

Statistical analysis: The materials of the research were processed in IBM SPSS 21.0 program (Chicago, IL, USA) of statistical analysis. The analysis of quantitative indicators distribution's correspondence to the law of normal distribution was checked by Shapiro-Wilk test (W). Mean value ± standard deviation (±SD) were calculated for the results of indicators that corresponded to the law of normal distribution. Median value (Me) and upper and lower quartiles (25%; 75%) were calculated for the indicators with a non-normal distribution. Student's t-test (for independent groups) was used to measure the significance of the difference, provided there was a normal distribution of study results; Mann-Whitney U test (for independent groups) and χ^2 criterion were used provided the indicators had a distribution other than normal.

RESULTS

CG included 13 males and 16 females, whereas MG included 16 males and 13 females ($p>0.05$). The mean age of CG patients was 69.3±9.77 years, and the mean age of MG patients was 67.9±10.46 years ($p>0.05$). During first examination of CG, 5 patients had satisfactory health condition, 12 patients had health condition close to satisfactory, 6 patients had moderately severe condition, and 6 patients had severe health condition. Among MG patients, 6 patients had satisfactory health condition, 15 patients had health condition close to satisfactory, 5 patients had moderately severe condition, and 3 patients had severe health condition. Statistical analysis did not reveal any significant difference in this distribution of patients ($p>0.05$).

According to the NIHSS Scale, the average value in CG was 8.41±2.31 with Me (25%; 75%) indicator being 9 (7;

Table I. Initial Fugl-Meyer scores of sensorimotor recovery in groups of patients

	Items	CG (n=29)		MG (n=29)	
		$\bar{x}$±SD	Me (25%; 75%)	$\bar{x}$±SD	Me (25%; 75%)
Motor function	Proximal part of the arm	11.10 ± 1.82	11 (9.5; 12.5)	11.31 ± 1.79	12 (10; 12.5)
	Hand and radiocarpal joint	5.07 ± 1.10	5 (4; 6)	5.07 ± 1.22	5 (4; 6)
	Total score of the upper limb	16.17 ± 2.87	16 (13.5; 18.5)	16.38 ± 2.93	17 (14; 18)
	Balance	6.03 ± 0.91	6 (5; 7)	6.24 ± 0.91	6 (6; 7)
Proprioceptive sensitivity	Shoulder joint	0.86 ± 0.58	1 (0,5;1)	0.93 ± 0.37	1 (1; 1)
	Elbow joint	0.79 ± 0.49	1 (0,5; 1)	0.83 ± 0.53	1 (0.5; 1)
	Radiocarpal joint	0.72 ± 0.53	1 (0; 1)	0.76 ± 0.56	1 (0; 1)
	Fingers	0.76 ± 0.58	1 (0; 1)	0.83 ± 0.60	1 (0; 1)
	Thumb	0.86 ± 0.64	1 (0; 1)	0.90 ± 0.49	1 (1; 1)
	Total sensitivity of the upper limb	10.03 ± 1.97	10 (8.5; 12)	10.48 ± 1.86	11 (9; 12)

CG – control group; MG – main group.

Table II. Final Fugl-Meyer scores of sensorimotor recovery in groups of patients

	Items	CG (n=29)		MG (n=29)	
		$\bar{x}$±SD	Me (25%; 75%)	$\bar{x}$±SD	Me (25%; 75%)
Motor function	Proximal part of the arm	22.81 ± 5.42	24 (18; 27)	27.72 ± 5.00	30 (25; 31.5)**
	Hand and radiocarpal joint	16.53 ± 4.20	17 (12; 21)	20.00 ± 4.69	21 (15; 24.5)**
	Total score of the upper limb	39.34 ± 8.36	41 (33.5; 47)	47.72 ± 8.81**	49 (45; 54.5)
	Balance	9.78 ± 1.72	11 (8; 11)	10.79 ± 2.30	12 (9.5; 12.0)**
Proprioceptive sensitivity	Shoulder joint	1.52 ± 0.51	2 (1; 2)	1.83 ± 0.38	2 (2; 2)*
	Elbow joint	1.66 ± 0.48	2 (1; 2)	1.72 ± 0.45	2 (1; 2)
	Radiocarpal joint	1.55 ± 0.51	2 (1; 2)	1.59 ± 0.50	2 (1; 2)
	Fingers	1.31 ± 0.47	1 (1; 2)	1.59 ± 0.50	2 (1; 2)*
	Thumb	1.34 ± 0.48	1 (1; 2)	1.62 ± 0.49	2 (1; 2)*
	Total sensitivity of the upper limb	13.36 ± 3.10	13 (11; 16)	16.29 ± 3.06**	16 (15; 18)

CG – control group; MG – main group; * – the difference between the indicators is statistically significant as compared to control group $p<0.05$; ** – $p<0.01$.

10) points; corresponding indicators in MG were 8.90±2.27 points and 9 (7.5; 10) points respectively. No statistical difference between the groups was revealed ($p>0.05$).

According to the Glasgow Coma Scale, Me (25%; 75%) indicator comprised 13 (10; 13) points in CG, and 12 (10.5; 13) points in MG ($p>0.05$).

It should be noted that Albert's Test scores in CG and MG were not statistically different at the first examination: 18.28±4.82 points in CG; 18.41±4.31 points in MG out of a maximum of 39 points.

Sensorimotor recovery indicators on the Fugl-Meyer scale in CG and MG had no statistical difference at the first examination (Table I). It should be noted that all measured indicators of sensorimotor recovery in CG and MG had significant improvements since the first examination ($p<0.01$).

According to the Fugl-Meyer scores of sensorimotor recovery at the final examination, ±SD indicator of the motor function of the proximal part of the arm in CG comprised 22.81±5.42 points out of 36 points possible, with Me (25%; 75%) indicator being 24 (18; 27) points. MG indicator was slightly higher and comprised 27.72±5.00 points, with Me (25%; 75%) indicator being 30 (25; 31.5) points. The difference between groups of patients at the final examination was proved significant ($p<0.01$). As for increase, it comprised 16.41 points in MG and 12 points in CG. Mean value of MG was 4.91 points higher, which comprised 21.5% of the final CG indicator.

At the final examination, the average score of the motor function of the hand and radiocarpal joint in CG comprised 16.53±4.20 points out of 30 points possible, with Me (25%; 75%) indicator being 17 (12; 21) points (Table 2). MG patients showed slightly better ±SD indicator, which comprised 20.00±4.69 points; Me (25%; 75%) indicator was significantly better ($p<0.01$) comprising 21 (15; 24.5) points. increase was bigger in MG and comprised 14.93 points, whereas in CG it comprised 11.47 points. Mean

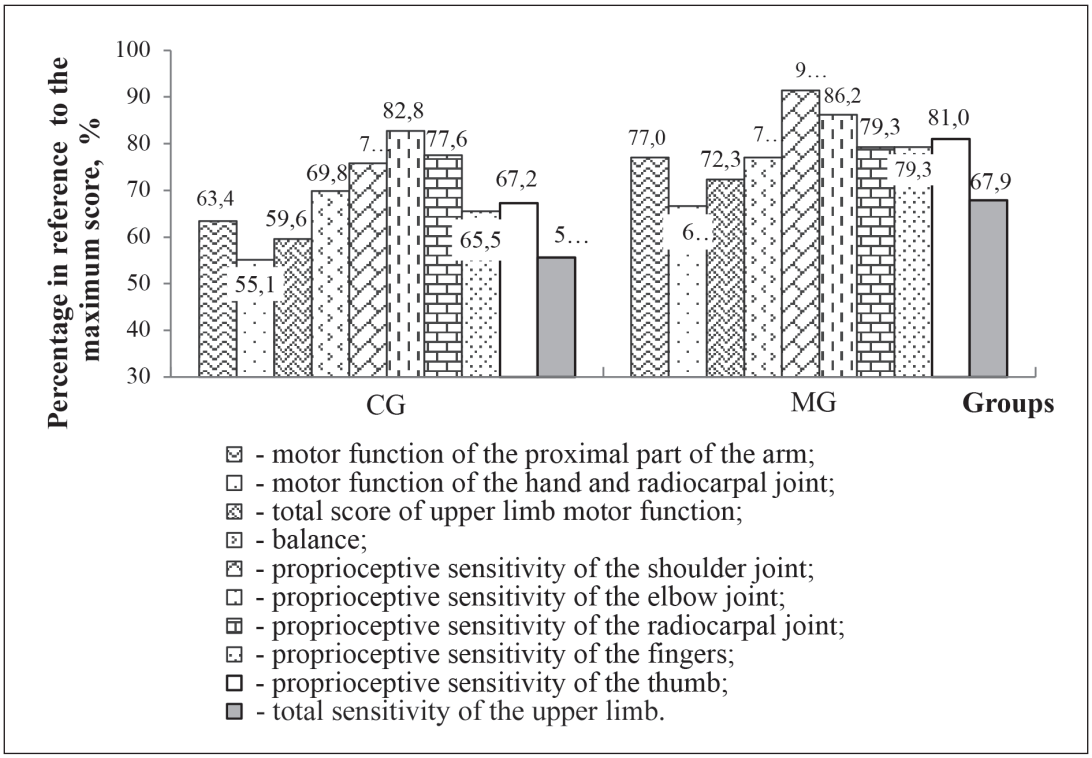

Fig. 1. Comparative final results of the assessment of indicators on the Fugl-Meyer scale in the main (MG) and control (CG) groups of patients

value of MG was 3.47 points higher, which comprised 21% of the final CG indicator.

The total score of upper limb motor function was significantly better in MG ($p<0.01$). MG mean value increased by 31.48 points and amounted to 47.72±8.81 points; CG mean value increased by 23.29 points and amounted to 39.34±8.36 points. Thus, MG mean value was 8.38 points higher, which comprised 21.3% of the final CG indicator.

Balance score on the Fugl-Meyer Scale was statistically better in MG when comparing the final scores (Table II). Me (25%; 75%) indicator comprised 12 (9.5; 12.0) points in MG and 11 (8; 11) points in CG ($p<0.01$). Mean values increased from 6.24±0.91 points to 10.79±2.30 points in MG and from 6.03±0.91 points to 9.78±1.72 points in CG. Thus, the increase in the groups comprised 4.55 and 3.74 points respectively. MG mean value was 1.01 points higher, which comprised 10.3% of the final CG indicator.

According to the Fugl-Meyer scores of sensorimotor recovery at the final examination, ±SD indicator of shoulder joint proprioceptive sensitivity in CG comprised 1.52±0.51 points out of 2 points possible, with Me (25%; 75%) indicator being 2 (1; 2) points. MG patients showed slightly higher results which comprised 1.83±0.38 points, with Me (25%; 75%) indicator being 2 (2; 2) points. The difference between the group scores at the final examination was proved significant ($p<0.05$). As for increase, it comprised 0.90 points in MG, and 0.66 points in CG. MG mean value was 0.31 points higher, which comprised 20.4% of the final CG indicator.

According to the analysis of the final examination scores of elbow joint proprioceptive sensitivity in CG, the average score comprised 1.66±0.48 points out of 2 points possible; Me (25%; 75%) indicators comprised 2 (1; 2) points. MG patients showed slightly better ±SD indicator, which amounted to 1.72±0.45 points, whereas Me (25%; 75%) indicators were similar and had no statistical difference ($p>0.05$). increase comprised 0.90 points in MG and 0.86 points in CG.

The absence of a significant difference in the final scores was also revealed when comparing radiocarpal joint proprioceptive sensitivity in MG and CG ($p>0.05$). Final Me (25%; 75%) indicators comprised 2 (1; 2) points in the groups. ±SD indicator increased from 0.72±0.53 points to 1.55±0.51 points out of 2 points possible in CG and from 0.76±0.56 to 1.59±0.50 points in MG. increase comprised 0.90 points in MG and 0.86 points in CG.

Fingers proprioceptive sensitivity was statistically better in MG. Me (25%; 75%) indicator was lower in CG comprising 1 (1; 2) points; Me (25%; 75%) indicator in MG comprised 2 (1; 1) points ($p<0,05$). ±SD indicator increased from 0.76±0.58 points to 1.31±0.47 out of 2 points possible points in CG and from 0.83±0.60 to 1.59±0.50 points in MG. increase comprised 0.76 points in MG and 0.55 points in CG. MG mean value was 0.28 points higher, which comprised 21.4% of the final CG indicator.

At the final examination, the average score of thumb proprioceptive sensitivity comprised 1.34±0.48 points out of 2 points possible in CG; Me (25%; 75%) indicator comprised 1 (1; 2) point. MG patients showed slightly better ±SD indicator which amounted to 1.62±0.49 points; Me (25%; 75%) indicator was statistically better ($p<0.05$) comprising 2 (1; 2) points. increase was higher in MG comprising 0.72

points, whereas in CG it comprised 0.48 points. MG mean value was 0.28 points higher, which comprised 20.9% of the final CG indicator.

According to the Fugl-Meyer scores of sensorimotor recovery at the final examination, ±SD indicator of the total sensitivity comprised 13.36±3.10 points out of 24 points possible in CG. The indicator was slightly higher in MG comprising 16.29±3.06 points. The difference between the group scores at the final examination was proved significant ($p<0.01$). As for increase, it comprised 5.81 points in MG and 3.33 points in CG. MG mean value was 2.93 points higher, which comprised 21.9% of the final CG indicator.

Taking into account that Fugl-Meyer scale indicators had different maximum scores, it is necessary to present final indicators in reference to maximum scores (Fig. 1). The analysis of the obtained results revealed that the maximum percentage was appointed to proprioceptive sensitivity of the shoulder joint (91.7% out of the maximum score) in MG, and to proprioceptive sensitivity of the elbow joint (82.8% out of the maximum score) in CG.

On the other hand, the minimum percentage in MG and CG was appointed to the motor function of the hand and radiocarpal joint (66.7% and 55.1% out of the maximum score, respectively).

DISCUSSION

First examination of the patients revealed low indicators of upper limb sensorimotor recovery according to the Fugl-Meyer scale. During the course of physical therapy and ergotherapy programs MG and CG patients showed significant improvements of all indicators and total scores of motor function and proprioceptive sensitivity.

At the same time, comparison of the final scores of the samples revealed a number of statistical differences in favor of MG patients, which confirmed the benefits of the combined program of physical therapy and ergotherapy they received.

It should be noted that according to the comparative analysis of final indicators, the largest difference between CG and MG was revealed in shoulder joint proprioceptive sensitivity indicator (15.5% out of the maximum theoretical score). The least difference between the groups was revealed in radiocarpal joint proprioceptive sensitivity indicator (1.7% out of the maximum score). A fairly small difference was revealed in elbow joint proprioceptive sensitivity indicator – 3.4% out of the maximum score. Both these indicators had no statistical advantages in any of the samples. In other items of the scale relative advantage (presented in % out of the maximum possible value) of CG over MG was as follows: motor function of the proximal part of the arm was 13.6; motor function of the hand and radiocarpal joint was 11.6%; total score of upper limb motor function was 12.7%; balance was 7.3%; proprioceptive sensitivity of the fingers was 13.8%; proprioceptive sensitivity of the thumb was 13.8%; total sensitivity of the upper limb was 12.2%.

Thus, the study confirmed positive impact of the combined program of physical therapy and ergotherapy carried out with MG patients on upper limb sensorimotor recovery.

Scientific literature presents studies that both confirm and refute the efficacy of certain interventions for upper limb sensorimotor recovery.

Yun G.J. et al. [20] studied the synergistic effect of mirror therapy and neuromuscular electrical stimulation (NMES) on arm function in stroke patients. Mirror therapy and NMES group patients showed significant improvements in hand, wrist, coordination, and hand extension strength indicators on the Fugl-Meyer scale as compared to the groups receiving only one of the therapies.

At the same time, a blinded randomized controlled trial [21] showed no evidence of mirror therapy efficacy in the early post-stroke period. In this study, the experimental group received mirror therapy, while the control group received sham therapy.

The comparison of impact of movement-based (MMT) and task-based mirror therapies (TMT) on upper limb function improvement in stroke patients confirmed the efficacy of both interventions in patients with mild and moderate hemiplegia caused by stroke. However, according to the study, MMT is more effective than TMT in improving function of hemiplegic upper limbs [22].

The positive effect on fine motor skills recovery with the help of a sensor glove was presented in the study of Prokopenko S.V. et al. [12]. While training, the patient used finger movements to play a computer game. The results of using a sensor glove indicate that a higher level of daily functioning has been achieved after the additional use of sensor glove as a rehabilitation method. Besides, this method is interesting for the patient, improves motivation to exercise involving the patient in the treatment process.

According to the results of the research, combination of ergotherapy and physical therapy is one of the best means for the recovery of post-stroke patients. Ergotherapy has a positive impact on the degree of recovery of certain functions in post-stroke patients, as well as on the level of their independence while performing all major household activities. Rational use of ergotherapy accelerates the recovery of muscle strength, normal joint range of motion, coordinated movements [23].

The study of Park S.E. [24] confirmed the benefits of combining proprioceptive neuromuscular facilitation and oculo-motor exercise for balance indicators recovery in patients with neglect as compared to interventions based on oculo-motor exercise alone or a combination of oculo-motor exercise with functional electrical stimulation.

Our study also confirmed better efficacy of the physical therapy program, which included proprioceptive neuromuscular facilitation.

Positive effect of a scanning and cueing approach (active scanning to the left was encouraged by the therapist, using visual and verbal cues and a mental imagery technique, during reading and copying tasks and simple board games) and a contralesional limb activation approach (functional and goal-oriented left upper-limb activities in neglected hemispace were encouraged) on

the results of star cancellation test, the line bisection test, and the baking tray task, conducted with patients having neglect syndrome, are presented in the study of Bailey M.J. et al. [16-3]. These results are generally consistent with ours.

Paolucci S. [25] is reported about motor and functional recovery of stroke patients with neglect. Patients were assessed by means of three functional and neurological scales (Rivermead Mobility Index, Barthel Index, Canadian Neurological Scale). Outcomes were significantly improved by the simultaneous presence of a treatment specifically focused on neglect.

Thus, the study confirmed that patients with neglect need special additional interventions within the physical therapy program, as well as better efficacy of therapy combinations, namely proprioceptive neuromuscular facilitation, oculo-motor exercise and visual tracking.

CONCLUSIONS

Statistical analysis of the Fugl-Meyer scale indicators confirmed that MG had statistical advantages in all studied items of the motor function domain (proximal part of the arm, hand and radiocarpal joint and total score of upper limb), as well as in a number of proprioceptive sensitivity indicators (shoulder joint, fingers, thumb and total sensitivity of upper limb). These results confirm better efficacy of the physical therapy program received by MG patients. In particular, final scores of motor function of the hand and radiocarpal joint comprised 16.53±4.20 points out of 30 points possible in CG and 20.00±4.69 points in MG ($p<0.01$).

REFERENCES

1. Kovalchuk V.V., Gusev A.O., Minnullin T.I. et al. Reabilitatsiya patsiyentov posle insul'ta. Kriterii effektivnosti i faktory uspekha: rol' fizicheskoy, neyropsikhologicheskoy i medikamentoznoy terapii [Post-Stroke Rehabilitation. Efficacy Criteria and Success Factors: a Role of Physical, Neuropsychological and Drug Therapy]. Effective Pharmacotherapy. 2017;(19):62-72. (in Russian).
2. Zozulya I.S., Golovchenko Yu.I., Zozulya A.I. et al. Zahal'ni pryntsypy diahnostyky, formuvannya diahnozu, likuvannya ta prohnozuvannya insul'tu [General principles of diagnostic, forming of diagnosis, treatment and prediction of stroke]. Ukrainian Med J. 2015;5(109):34-8. (in Ukrainian).
3. Zakaliak N.R., Sprynska O.M. Rol' likuval'noyi himnastyky u vidnovlenni rukhovoyi funktsiyi verkhnikh kintsivok u khvorykh na pislyainsul'tnyy hemiparez [The role of therapeutic gymnastics in the restoration of motor function of the upper extremity in patients with post-stroke hemiparesis]. In: Kopytina YaM, Liannoi MO, editors. Proceedings of the III All-Ukrainian Correspondence Scientific and Practical Internet Conference Health problems, physical therapy, rehabilitation and occupational therapy; 2017 Dec 21; Sumy, Ukraine. Sumy: AS Makarenko SUMPU. 2017: 65-8. (in Ukrainian).
4. Mytskan B., Edynak G., Ostapyak Z. et al. Insul't: prychyny, faktory ryzyku, fizychna reabilitatsiya [Stroke: Causes, Factors of Risk, Physical Rehabilitation]. Physical education, sport and health culture in modern society. 2012;(19):295-302. (in Ukrainian).
5. Russkikh O.A., Perevoshchikov P.V., Bronnikov V.A. Sindrom ignorirovaniya (neglekta) u postinsul'tnykh bol'nykh i vozmozhnosti neyropsikhologicheskoy reabilitatsii [The syndrome of ignorance (neglect) in post-stroke patients and the possibility of neuropsychological rehabilitation]. In: Krasnoryadtseva OM, editor. Proceedings of the VII Siberian Psychological Forum Complex human studies: psychology; 2017 Nov 28-29; Tomsk, Russia. Tomsk: TSU. 2017; 1: 127-31. (in Russian).
6. Heilman K.M., Valenstein E. Frontal lobe neglect in man. Neurology. 1998;50(5):1202-2-a.
7. Damasio A.R., Damasio H., Chui H.C. Neglect following damage to frontal lobe or basal ganglia. Neuropsychologia. 1980;8:23-32.
8. Mironenko T.V., Vasilenko V.N., Kovalik V.S. Istoriya bolezni: Neglekt-sindrom u patsiyentov, perenesshikh kardioembolicheskiy insul't [Case records: Neglekt-syndrome in patients undergoing cardioembolic stroke]. University clinic. 2017;13(1):118-22. (in Russian).
9. Evzelman M.A. Narushenie rechi u bolnyih s mozgovyim insultom i ee korrektsiya [Speech impairment in patients with cerebral stroke and its correction]. Orel. 2005:112. (in Russian).
10. Kuznetsova S.M., Iegorova M.S. Sovremennyye predstavleniya o kardioembolicheskom insul'te [Current understanding of cardioembolic stroke]. Doctor. 2003;(3):13-6. (in Russian).
11. Kalinina S.Ya., Semenova T.N., Grigoryeva V.N. Disfunktsiya verkhnikh konechnostey u patsiyentov s insul'tom [Upper limb dysfunction in stroke patients]. Practical medicine. 2017;1(102):40-4. (in Russian).
12. Prokopenko S.V., Mozheyko E.Yu., Alexeevich G.V. Vozmozhnost' vosstanovleniya povyshennoy aktivnosti ruk s pomoshch'yu sensornoy perchatki u patsiyentov s insul'tom [The possibility of restoring the advanced hand activity using the sensorial glove in patients with stroke]. Siberian Medical Review. 2014;(2):72-7. (in Russian).
13. Khizhnikova A.E., Klochkov A.S., Kotov-Smolensky A.M. et al. Dvigatel'noye obucheniye patsiyentov s postinsul'tnym parezom ruki na mekhanoterapevticheskom komplekse [Motor learning of the post-stroke patients presenting with upper limb paresis on the mechanotherapeutic system]. Problems of Balneology, Physiotherapy, and Exercise Therapy. 2018;95(1):20-5. (in Russian).
14. Simpson L.A., Eng J.J. Functional recovery following stroke: capturing changes in upper-extremity function. Neurorehabil Neural Repair.2013;27(3):240-50.
15. Kim Y.M., Chun M.H., Yun G.J. et al. The effect of virtual reality training on unilateral spatial neglect in stroke patients. Ann Rehabil Med. 2011;35(3):309-15.
16. Bailey M.J., Riddoch M.J., Crome P. Treatment of visual neglect in elderly patients with stroke: a single-subject series using either a scanning and cueing strategy or a left-limb activation strategy. Phys Ther. 2002;82(8):782-97.
17. Paolucci S., Antonucci G., Grasso M.G. et al. The role of unilateral spatial neglect in rehabilitation of right brain-damaged ischemic stroke patients: a matched comparison. Arch Phys Med Rehabil. 2001;82(6):743-9.
18. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191-4.
19. Verkhovna Rada of Ukraine. Osnovy zakonodavstva Ukrainy pro okhoronu zdorovia Zakon Ukrayiny 1992, Z. No. 2802-XII [Fundamentals of the Ukrainian Legislation on Health Care Act of 1992, L. No. 2802-XII]. Kyiv: Vidomosti Verkhovnoi Rady Ukrainy [updated 2020 Jun 24; cited 1993 Nov 19]. https://zakon.rada.gov.ua/laws/show/2801-12#Text . (in Ukrainian).

20. Yun G.J., Chun M.H., Park J.Y. et al. The synergic effects of mirror therapy and neuromuscular electrical stimulation for hand function in stroke patients. Ann Rehabil Med. 2011;35(3):316-21.
21. Antoniotti P., Veronelli L., Caronni A. et. al. No evidence of effectiveness of mirror therapy early after stroke: an assessor-blinded randomized controlled trial. Clin Rehabil. 2019;33(5):885-93.
22. Bai Z., Zhang J., Zhang Z. et al. Comparison Between Movement-Based and Task-Based Mirror Therapies on Improving Upper Limb Functions in Patients With Stroke: A Pilot Randomized Controlled Trial. Front Neurol. 2019;10:288.
23. Freburger J.K., Dongmei Li., Fraher E.P. Community Use of Physical and Occupational Therapy After Stroke and Risk of Hospital Readmission Arch Phys Med Rehabil. 2018;99(1):26-34.e5. doi: 10.1016/j.apmr.2017.07.011.
24. Park S.E., Min K.O., Lee S.B. et al. Effect of eye movements and proprioceptive neuromuscular facilitation on balance and head alignment in stroke patients with neglect syndrome. J Phys Ther Sci. 2016;28(2):596-601.
25. Paolucci S., Antonucci G., Guariglia C. et al. Facilitatory effect of neglect rehabilitation on the recovery of left hemiplegic stroke patients: a cross-over study. J Neurol. 1996;243(4):308-14.

ORCID and contributionship:
Olena A. Dulo: 0000-0003-0473-5605 [A,B,E,F]
Yurii M. Dido: 0000-0002-1728-4025 [A,C,D,E]

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CORRESPONDING AUTHOR
Yurii M. Dido
Uzhhorod National University
3 Narodna Square, 88000 Uzhhorod, Ukraine
tel: +380509222710
e-mail: yura.dido.1994@gmail.com

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ORIGINAL ARTICLE

TRANSCATHETER VERSUS SURGICAL AORTIC VALVE REPLACEMENT: FIRST EXPERIENCE WITH A NEW TAVI SYSTEM

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Glib I. Yemets, Oleksandra V. Telehuzova, Andrii V. Maksymenko, Georgiy B. Mankovsky, Yevhen Y. Marushko, Arkadii A. Dovgalyuk, Illya M. Yemet
UKRAINIAN CHILDREN'S CARDIAC CENTER, KYIV, UKRAINE

ABSTRACT

The aim: to reveal early results after transapical TAVI with a new self-manufactured XPand system, comparing them with SAVR and common transfemoral TAVI outcomes.

Materials and methods: Eighty-four patients (mean age 79,5±10,2 years) with severe aortic stenosis were operated on from January 2016 to February 2019. Nine patients had undergone the TAVI (two with transfemoral access route and seven with transapical, using the XPand system). SAVR was performed in seventy five patients. For the latter, we estimate the in-hospital mortality, complication rates, intensive care unit and total hospital length of stay.

Results: There was no intraoperative mortality. In the TAVI group, the frequency of intraoperative and postoperative complications was significantly lower ($p<0.01$). The SAVR group showed higher median intensive care unit length of stay (104 h, IQR 72 –112 versus 29 h, IQR 20–35,$p<0.01$), hemodynamic support duration (100,98 ± 78 minutes versus 11.13 ± 7.89 minutes, $p<0.01$) and paravalvular leakage causality (9,33% versus 0%). No significant difference in results depending on the TAVI access routes was obtained.

Conclusions: We conclude that TAVI provides an alternative to the conventional approach in patients with severe aortic stenosis aged over 75 years. No significant difference in mortality rate between TAVI and SAVR groups was found. A novel transapical TAVI device is associated with good short-term results and lower complication rate.

KEY WORDS: transcatheter aortic valve replacement, TAVI, SAVR, aortic stenosis, elderly patients, XPand

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INTRODUCTION

Cardiovascular disease (CVD) is of interest because supposed to be the most common source of adult morbidity and mortality for many decades [1]. Among the pathology variety, leading position is traditionally occupied by the coronary heart disease, acquired valvular heart disease and other conditions provokingthe life expectancy reduction, especially in the elderly [2]. With a dramatic geriatric population life expectancy increase in Western Europe and North America, acquired defects due to degenerative valve changes, came to the fore in the valvular pathology structure beside adults. These rapid changes are having a serious effect on treatment approaches and strategies, pushing the process of knowledge producing.

Nevertheless, degenerative aortic stenosis (AoS) is the most common valve disease in adults, whose prevalence increases with age, affecting approximately 4% of individuals older than 75 years [3]. A primary concern of AoS is unfavourable prognosis with symptoms manifestation and conservative treatment. Therefore, the average life expectancy after the anginal attacks, syncope or heart failure appeared in these patient groups is only 5, 3 and 2 years, respectively [4].

Surgical aortic valve replacement (SAVR) is a traditional method which has considerable impact on life quality and duration. However, approximately 30% of patients with AoS and surgical indication are not submitted to valve replacement due to advanced age, presence of comorbidities, high surgical risk etc. Osama Alsara et. al [5] notes that advanced age has been a leading barrier to surgical intervention in elderly patients based on the increasing with age peri-operative mortality rate from 1.3% in patients ≤ 70 years old, to about 5% at age 80-85 years. Aforementioned statements turning out the benefit / risk surgical replacement ratio for being controversial.

More than a decade ago, the transcatheter aortic valve implantation (TAVI) was offered as an alternative option. Describing this procedure, we have to admit that traditionally prosthetic valve insertion is performed through the vascular access and withdraw the need for open heart surgery (artificial circulation and cardiac arrest are not required). Thus, the periprocedural death, risk of bleeding and other complications could be reduced. Although TAVI is widely used for symptomatic severe aortic stenosis in patients over 75 in Europe and North America. Alas, in Ukraine, this procedure had been performed only in a few isolated cases [6].

As discussed earlier, to manage the severe aortic stenosis two major approaches are usually applicable: TAVI and SAVR. These methods have achieved significant improvements in survival rates as well as other outcomes in patients with severe aortic stenosis [7]. However, approximately 5.2% among elderly patients with symptomatic severe aortic stenosis undergoing surgical aortic valve

Table I. Baseline patients characteristics in both groups

	TAVI (mean±SD)	SAVR (mean±SD)	p-value
Number	9	75	
Age,years	81 ± 8 ,2	78.53 ± 1.7	<0.01
Male	1 (11.1%)	40 (53.3%)	< 0.001
Coronary Heart Disease	5 (55.5%)	63 (84%)	< 0, 05
Multivascular lesions	0	45 (60%)	<0.001
NYHA III, IV	8 (88.9%)	54 (72%)	< 0 , 01
AF before surgery	0	15 (20%)	<0, 0 01
COPD	0	4 (5.3%)	<0.05
Diabetes	2 (22.2%)	30 (40%)	<0.05
EuroScore II	9.79 ± 5.1	9.41 ± 4.06	= 0.1
STS Score	7.85 ± 2.35	7.62 ± 3.24	= 0.1

replacement are in high-risk and in 80% cases they meet the criteria for TAVI. The distribution of these indicators to the general population in Western Europe and North American countries shows that approximately 290 thousand adults in mentioned regions are potential candidates for TAVI [6,7]. Extrapolating given by Osnabrugge et al [8] data to the Ukrainian real life population over 75 (rough quantity in 2013 was about 3 million), the number of symptomatic aortic stenosis patients considered to be candidates for TAVI could vary to 14 thousands. These theses highlight the importance of transcatheter aortic valve replacement in Ukraine.

Annually, we perform more than 25 procedures on patients with aortic stenosis over 75 in our Clinic. There is a growing body of literature that recognises the importance of managing these patients primarily associated with comorbidity and severe general condition, therefore with a very high risk of surgery due to the artificial circulation [9]. The specific objective of this study was to present the primary data pool on the aortic stenosis treatment by both TAVI and SAVR, and to analyze the immediate and long-term outcomes of described methods. Obtained data should be generalized, according to the hight external validity.

THE AIM

The aim was to reveal early results after transapical TAVI with a new self-manufactured XPand system, comparing them with SAVR and common transfemoral TAVI outcomes.

MATERIALS AND METHODS

Study was performed according to the Good Clinical Practice (GCP) standards and World Medical Association (WMA) Helsinki Declaration. Prior to participation and data collection, all subjects received an explanation of the project and signed the inform consent.

An observational and longitudinal study was carried out at Ukrainian Children's Cardiac Center. Clinic for Adults (UCCC) located in Kyiv. We include all patients over 75 with severe, symptomatic AoS, submitted to TAVI or SAVR, from January 2016 to February 2019. The participant's group was representative with respect to gender and age. In order to achieve valid results, we analysed anamnesis of patients, treatment protocols and early postprocedural follow – ups of 84 aortic valve replacement operations. Preprocedural data included the clinical examination and complementary tests (resting electrocardiography, chest X-ray, transthoracic Doppler echocardiography with protocol for aortic complex measurements; computed tomography (CT) angiography of the heart and total aorta, and cardiac catheterization with coronary angiography). Transesophageal echocardiography was used to guide the procedure and detect possible complications due to prosthesis implantation. In some cases, additional tests were performed to rule out or confirm comorbidity (MRI of the abdominal cavity, etc.). Patients, undergoing TAVI were additionally examined by MRI with contrast to assess femoral and superficial arteries. Among the analyzed cases, 75 procedures (89.28%) were SAVR and 9 (10,7%) – TAVI.

ELIGIBILITY CRITERIA

Inclusion criteria for transcatheter aortic valve implantation were: (1)severe aortic stenosis, (2) valve's calcification or fibrosis and (3) age > 75 years.

Exclusion criteria were: (1) multivessel hemodynamically significant coronary artery disease required surgical correction, (2) the distance from the aortic valve fibrous ring to the coronary arteries less than 10 mm, (3) severe mitral and tricuspid insufficiency.

GROUP CHARACTERISTICS

A number of patients with isolated aortic stenosis in TAVI group was 4 (44.4%). Just over half the sample (55,6%) was diagnosed with coronary heart disease, which is considered to be an aggravating factor and adversely affects the life expectancy prognosis. In SAVR group, 63 patients (84%)

were diagnosed with coronary heart disease and 24 patients (32%) with type II diabetes mellitus. The chronic obstructive pulmonary disease was diagnosed in 4 patients (5%). The primary baseline data are given in Table I.

The risk of surgery using EuroSCORE II and STS was calculated for each patient and showed higher values in TAVI group. The statistical significance was not estimated. However, each risk stratification model suffers from limitations and still not perfect [10]. Baseline echocardiographic parameters are shown in Figure 1.

STATISTICAL ANALYSIS

Continuous variables were described as mean and standard deviation, and categorical variables as absolute number and percentage. Statistical significance was analysed using analysis of variance and t-tests as appropriate. Student's t-test (or the Mann-Whitney test) was used to compare continuous variables for independent samples, and the chi-squared test or Fisher's exact test was used for categorical variables, as appropriate. To assessif the means of two groups are significantly different the ANOVA was used. All analyses were performed using Stata IC and Excel software version. Statistical significance level was set at 5%.

PATIENT PREPARATION AND TYPE OF PROSTHESIS USED

All TAVI patients were pretreated with 100mg acetylsalicylic acid and 300mg clopidogrel on the day prior to implantation. In patients with renal dysfunction, intravenous hydration with 0.9% saline solution at a dose of 0.3 to 0.5mL/kg/hour was started 12hours before the procedure.

The self-expanding XPand prosthesis was used for transapical access. Patients were admitted to the intensive care unit after the intervention, and on the first day after the procedure, underwent laboratory tests, electrocardiogram, and echocardiogram.

SURGICAL PROCEDURES

TAVI

The procedures were performed in a hybrid operating room (HOR) under general anaesthesia. Two access routes for TAVI implantation were performed: transapical andtransfemoral (7 procedures and 2 procedures, respectively) with mean duration 173.3 ± 35.5 min.

Essential Key Steps:

- To evaluate and confirm the transcatheter heart valve size by CT and ECHO
- To study the deployment side (comparing right or left femoral artery)
- Vascular access
- Place the transvenous right ventricle pacing catheter
- Sheath insertion
- To place the diagnostic pigtail catheter in the non-coronary cusp
- Establish coplanar view
- Cross the native valve
- To assess and stabilise hemodynamics
- To place the transcatheter heart valve
- Assess placement at 2/3 deployment (valve can be recaptured and repositioned at this point)
- Transcatheter heart valve full deployment
- Measuring the post-deployment
- Device and sheath removal
- Vascular closure

Transfemoral TAVIs were performed under the general anaesthesia with usage both fluoroscopic and ultrasound guidance to ensure the optimal percutaneous stick. Transfemoral arterial and venous accesses were obtained with 18F introducer, subsequently introducing the J -shaped wire and a pigtail catheter positioned in the ascending aorta. Safari wire was exposed to the left ventricle. The valve delivery system was loaded onto the guidewire with the flush ports pointing up. An angiogram was obtained to determine the appropriate TAVI deployment angles. Once the valve delivery system was brought into appropriate implanting position (depth of 3-5 mm), a coplanar image projection was achieved to see the radiopaque marker as a straight line. Additionally, the latter is preferred if both cannot be achieved together. In all cases of transfemoral implantation, we got good procedural outcome without complications.

Transapical TAVIs were done by the left-side thoracotomy through a 4-inch incision between the ribs. The left ventricular (LV) apex was exposed using a limited left anterolateral thoracotomy via ultrasound control. To deliver LV stimulation, the cathode of an external pacemaker was attached to the distal external end of the guidewire using a crocodile clip. A double purse-string suture with Teflon was placed into the left avascular zone.We punctured a LV apex with6F introducer, then inserted a soft conductor through the aortic valve to abdominal aorta bifurcation.The left ventricular apex was accessed, and a stiff wire was placed across the aortic valve to insert the 22F delivery sheath. A system for aortic valve ballooning was exposed via wire. Valve unfolding was carried out by transcatheter introduction of a balloon or self-expandable valve under high rate ventricular pacing (160–170 bpm) aimed to reduce heart movement. Heart Team assessed the intraoperative graft position, coronary arteries permeability and aortic valve function via angiography and ultrasound. Finally, the guidewire was removed, the sutures locked and cut.

In all transapical aortic valve implantation cases we used the self-expandable novel XPand prosthesis, created in collaboration between German, French and Ukrainian scientists. Device consists of two parts: (1) tubular slotted stainless-steel stent frame with unidirectional trileaflet bovine pericardial tissue and a (2)fabric skirt.Trileaflet bovine pericardial tissue sutured into a wire frame of nitinol, a nickel (55%)-titanium (45%) alloy that has temperature-associated shape memory features.

Currently, XPand device could be used only for the transapical access. It has been second standard approach

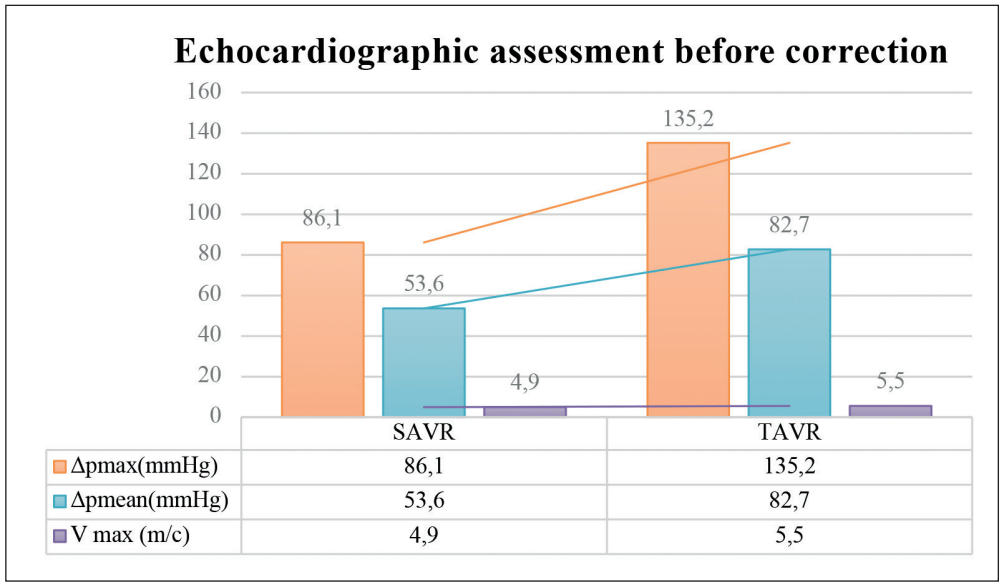

Fig. 1. Basic echocardiographic assessment in both groups before correction

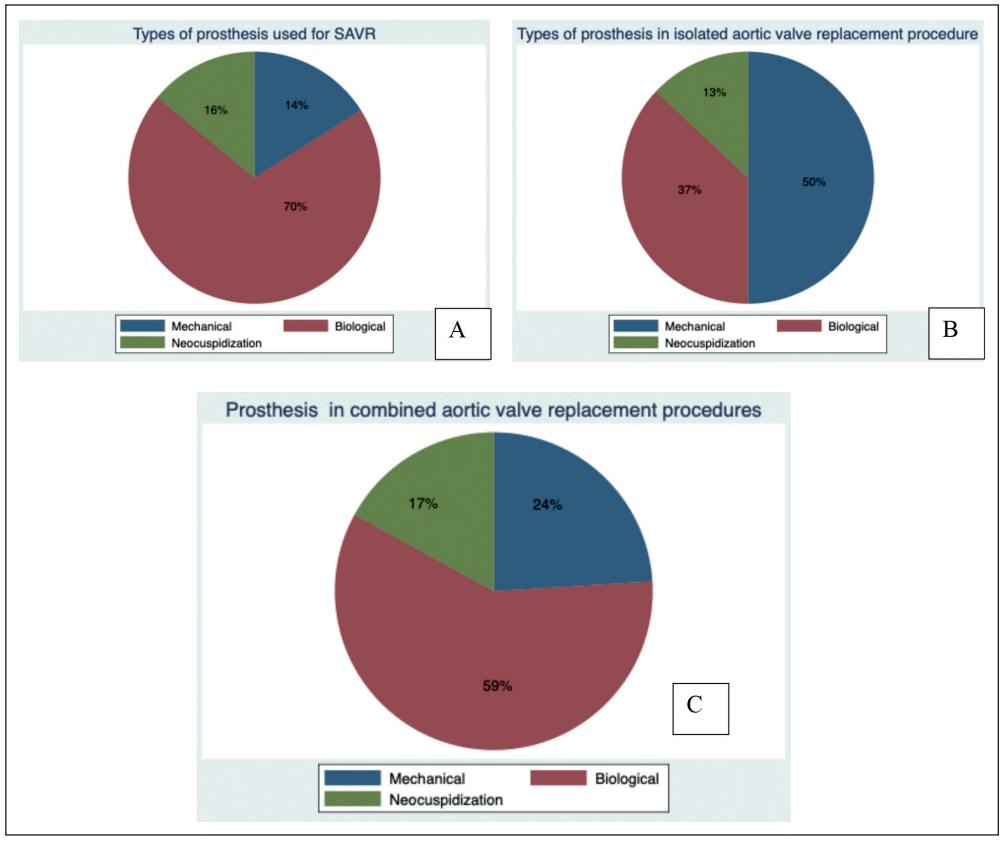

Fig. 2. Types of prosthesis used in SAVR group: . A – General data, B – For isolated aortic valve replacement, C – For combined procedures

after transfemoral, with stable rates although the advancement of alternative options [12]. Transapical access, aside from the ability to deliver a large device in a patient with inadequate iliofemoral vessels, may offer additional advantages in complex patients. Undoubtable advantage of this approach is the shortest and direct access to the aortic valve, which makes the procedure of prosthesis delivery and positioning more controllable.

SAVR

The procedures were performed with cardiopulmonary bypass (CPB) and moderate hypothermia (28-32o C) using anterograde crystalloid cardioplegia for myocardial protection. Patients underwent surgery through a median sternotomy, the heart was arrested after cardiopulmonary bypass. The participants were divided into two groups based on the type of procedure: isolated aortic valve replacement (n=15)

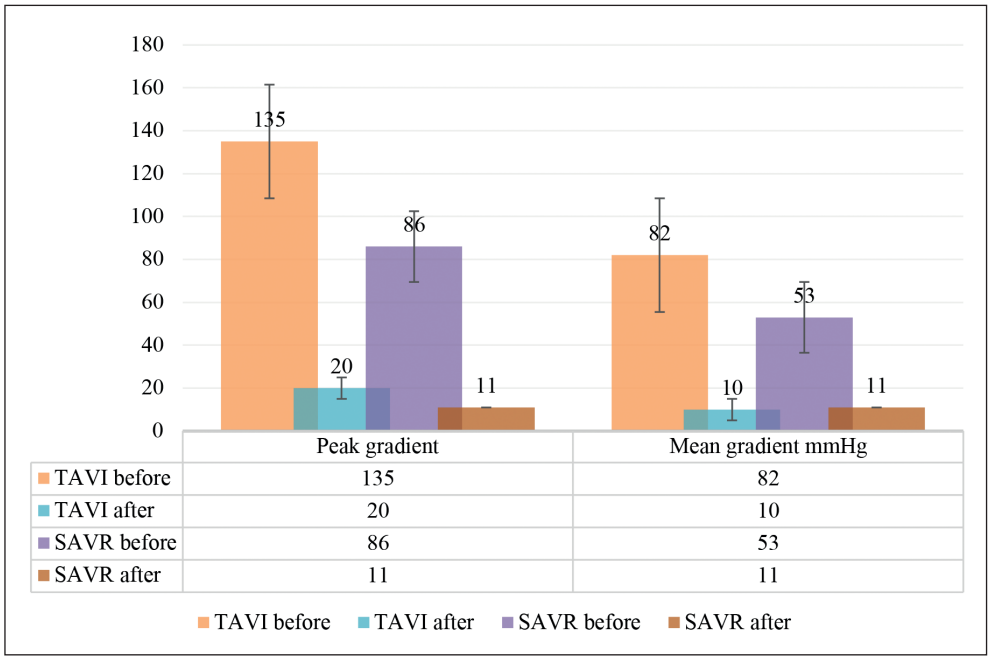

Fig. 3. Echocardiographic assessment before and after procedures

and coronary artery bypass grafting (CABG) with aortic valve replacement (n=41). The mean number of bypasses in combined procedures was 2.87 ± 0.66 per patient. We performed aortic root enlargement procedure described by Nicks et. al for five patients (6,67%). In52 (69,3%) cases AV valve was replaced with biological prosthesis, in 12 cases (17,4%) – with mechanical prosthesis and in ten cases (13,3%) aortic valve neocuspidization with autologous pericardium was performed. **Figure 2** presents the results obtained from the preliminary analysis of SAVR group.

RESULTS

PRIMARY OUTCOMES

It should be noted that all patients had a good postprocedural outcomes, confirmed by the obtained data. No critical complications requiring additional treatment (eg, ECMO and dialysis) have been identified.Strong evidence was found when comparing transfemoral versus transapical TAVI. Both accesses showed good results with no difference in 30-day mortality rates and ultrasound characteristics between groups. This is a remarkable result to conclud that transapical could be an alternative option for transfemoral access.

If we now turn to the average length of stay (ALOS) (from admission to discharge, transfer) ranged as 21.35 ± 8.1 days. The most striking result to emerge from the data is that average ICU length of stay (LOS), time of the artificial ventilation (ventilation) and duration of inotropic support were significantly lower in TAVI group. The differences between groups outcomes and complications are highlighted in Table II and III, respectively.

The next section of the survey was concerned with procedure time, which was significantly lower in TAVI group- 173.3 ± 22.4 min versus 226.7 ± 31.5 minin SAVR groupand duration of anaesthesia: 354.3 ± 26.5 min in TAVI group and 428±30.4 min in SAVR group. The mean intraoperative bleeding volumewas significantly lower in TAVI group, 208.8 ± 13.8 ml comparing with 978.5 ± 45.6 ml (p <0.01) in SAVR group.

Echocardiographic assessment of the procedure effect reliedon peak velocity, mean pressure gradient (MPG), aortic valve area (AVA) and left ventricle ejection fraction (EF). In the current study, comparing preprocedural echocardiographic assessment with postprocedural showed that the mean value of positive changes did not show any significant difference between TAVI and SAVR groups. The results obtained from the preliminary analysis are set out in Figure 2.

The single most striking observation to emerge from the data comparison was low level of paravalvular insufficiency in both groups. A minority of subjects in TAVI group (20,4%) indicated the minimal paravalvular insufficiency. However, 9% of patients in the surgical group were diagnosed with moderate paravalvular insufficiency, while patients in the TAVI group had no data for moderate paravalvular insufficiency. Figure 3.

Interestingly, there were also differences in the ratios of postcardiotomy syndrome frequency between two groups (p <0.01). Finally, important clinically relevant finding was that the postcardiotomy syndrome was mild and did not require additional manipulations (eg, pericardial drainage) in the TAVI group.

The syndrome was characterized by pleural effusions (up to 20 mm), increased levels of C-reactive protein and subfebrile, which were cured conservative. In contrast, in SAVR subjects, the duration of postcardiotomy syndrome and its severity prevailed over those in TAVI patients. The syndrome was also characterized by pericardial or pleuritic

Table II. Primary postprocedural outcomes in the TAVI and SAVR groups

	TAVI	SAVR	p value
Surgery duration, min	173.3 ± 35 , 5	354.2 ± 113.6	<0, 001
Average length of stay, days	24.1 ± 2.4	18.6 ± 3.2	<0.05
Average stay duration in ICU, days	1.29 ± 0.5	4.75 ± 2.89	<0.01
Ventilation duration, hours	12.01 ± 7.2	19.35 ± 9.68	<0.05
Hemodynamic support duration, hours	11.13 ± 7.89	100,98 ± 78 , 99	<0.001

Table III. Mortality rates and complications in the TAVI and SAVR groups

	TAVI	SAVR	p value
30-day mortality rate	0	3 (4%)	< 0, 1
Intraoperative blood loss, ml	208.8 ± 81.6	978.5 ± 230.3	<0.01
Blood loss by drainages, ml	130.5 ± 34.7	331.72 ± 120.8	<0.01
Resternotomy	0	4 (5.3%)	< 0.0 5
Pneumonia	0	9 (12%)	< 0, 001
Atrial fibrillation	3 (33.3%)	30 (40%)	<0.01
Paravalvular leakage	0	7 (9,33%)	<0.01
Postpericardiotomy syndrome	2 (22.2%)	23 (31.3%)	< 0.01
EX implantation	1 (11.1%)	6 (8%)	<0.05
Stroke	1 (11.1%)	2 (2.7%)	= 0.1
Sepsis	0	0	
Wound complications	0	5 (6.7%)	<0.05

pain, friction rubs, pleural effusions, pneumonitis, and abnormal ECG and radiography findings, required surgical manipulations (8% of patients underwent pericardial drainage). Surprisingly, TAVI was less common found to cause the atrial fibrillation – 33.3% compared to 46.3% in the SAVR group (p <0.01).

These findings will doubtless be much scrutinised, but there are some immediately dependable conclusions for 30-day mortality rates in patients of high surgical risk over 75, which didn't exceed 4% in our study. Recently, we can report the low mortality rate and it is apparent from the data, that 30-day mortality rate in TAVI group was 0 cases. The follow-up results showed that the annual mortality rate in patients after surgical aortic valve prosthesis was 2.6% (the cause of death was fatal comorbidities not related to thecardiovascular system).

The results in this chapter indicate that 12 months follow-up outcomes and survival analysis showed 2,6% mortality rate in SAVR group (however, due noncardiovascular diseases), simultaneously in TAVI group absence of death events was reported. The next chapter, therefore, moves on to discuss the pros and cons of TAVI for high risk patients over 75.

DISCUSSION

The empirical findings in this study provide a new understanding of TAVI and SAVR advantages. However, TAVI is an evolving modality currently available for patients with severe AS who are unsuitable for surgery because of technical/anatomical issues or too high-estimated surgical risk. This new understanding should help to improve predictions of the impact of transcatheter aortic valve implantation and to form generally new approach in AS treatment. The present study adds to the growing body of research that indicates TAVI competitive advantages in high surgical risk patients aged over 75, contemporaneously the "golden standard" – surgical valve replacement.

Prior studies that have noted the potential benefits of TAVI [14,15], taken together, results of our study suggest that this method provides less blood loss and complications, simultaneously reducing average stay duration in ICU.

Our study will prove useful in expanding the understanding of alternative to SAVR approach, which is the intervention of choice for older patients worldwide. The scope of this study was limited in terms of TAVI novelty for Ukraine, nevertheless, we were able to establish that both methods were safe and effective, as far as no significant difference in overall mortality rates between TAVI and SAVR was found. These results corroborate the findings of a great deal of the previous meta-analyzes [5,8,9].

According to the *SURTAVI* and *PARTNER* IIA trials data, we can infer that *safety* and *efficacy* of TAVI approach indicating the widespread use of this technique among high-risk patients [6,7].

Our study found that open surgical aortic valve replacement was associated with a higher surgery duration, increased bleeding risk and incidence of postoperative complications (pneumonia, postpericardiotomy syndrome and acute renal failure).

Several questions still remain to be answered, although the findings of this study have a number of practical implications.We found numerous advantages for TAVI in elderly patients:

1. Reduction of Intensive Care Unit length of stay.
2. Minimize blood loss.
3. Reduction of surgery duration.
4. Artificial blood circulation is not required.
5. Possibilities to perform TAVI in patients of high surgical risk with severe comorbidities and patients required re-intervention.

This research has thrown up many questions in need of further investigation.Numerous clinical challenges including paravalvular insufficiency, vascular complications, coronary artery occlusion, valve ring rupture, ventricular perforation, stroke etc. still remain unsolved. A reasonable approach to tackle this issue could be to manage this group of patients by the Heart Team [16].

Notwithstanding the relatively limited sample, this work offers valuable insights into the clinical features of AS patients over 75, presenting a novel XPand device. According to the high cost of TAVI, and therefore limited number of procedures associated with the rapidly increasing number of patients, Ukrainian scientists have developed a modern aortic valve biological prosthesis and delivery system. Undoubtedly, this invention is able to solve many issues with consistently high quality and reliability.

CONCLUSIONS

1. No significant difference in mortality rate between TAVI and SAVR groups was found.
2. The frequency of complications, including pneumonia ($p = 0.001$) and postcardiotomy syndrome ($p = 0.01$) was lower in TAVI group.
3. There was a statistically significant Reduction of Intensive Care Unit length of stay in TAVI group (<0.01).
4. No statistically significant difference between echocardiographic assessment was detected.
5. AF in TAVI patients has been significantly lower ($p <0.01$).

REFERENCES

1. Mallis P., Michalopoulos E., Dimitriou C. et al. Histological and biomechanical characterization of decellularized porcine pericardium. Bio-Medical Materials and Engineering. 2017; 28:477–488.
2. Hughes G.H., Cutter G., Donahue R. et al. Recruitment in the Coronary Artery Disease Risk Development in Young Adults (Cardia) Study. Control Clin Trials. 1987;8:68S–73S.
3. Vahanian A., Alfieri O., Andreotti F. et al. Joint Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology (ESC); European Association for Cardio-Thoracic Surgery (EACTS). Guidelines on the management of valvular heart (version 2012). Eur Heart J. 2012;33(19):2451-96.
4. Townsend C.M. Jr., Beauchamp R.D., Evers Mattox K.L. Sabiston textbook of surgery: the biological basis of modern surgical practice. Philadelphia. 2008;18.
5. Isarah A., Alsara O., Bachauwa G. Hypertension Management in the Elderly: What is the Optimal Target Blood Pressure? Heart Views. 2019;20(1) 11-16. doi:10.4103/heartviews.heartviews_28_18.
6. Siontis G.C.M., Overtchouk P. , Cahill T.J. et al. Transcatheter aortic valve implantation vs. surgical aortic valve replacement for treatment of symptomatic severe aortic stenosis : an updated meta-analysis. Eur Heart J. 2019 Oct 7;40(38):3143-3153. doi: 10.1093/eurheartj/ehz275.
7. Eghbalzadeh K., Rahmanian P.B., Giese D., Kuhn E.W. SAVR versus TAVI: What about the Hemodynamic Performance? An In Vivo and In Vitro Analysis. 2019.
8. Osnabrugge R.L., Kappetein A.P., Reynolds M.R., Cohen D.J. Cost-effectiveness of transcatheter valvular interventions: economic challenges. 2013.
9. Baumgartner H., Falk V., Bax J.J. et al. 2017 ESC / EACTS Guidelines for the management of valvular heart disease. Eur Heart J. 2017; 38: 2739–91.
10. Khan A.A., Murtaza G., Khalid M. F., Khattak F. Risk Stratification for Transcatheter Aortic Valve Replacement. Cardiology research. 2019;10(6):323–330. https://doi.org/10.14740/cr966.
11. Reardon M.J., VanMieghem N.M., Popma J.J. et al. Surgicalortranscatheteraortic-valvereplacementinintermediate-riskpatients. N Engl J Med. 2017;376:1321–31. https://doi.org/10.1056/NEJMoa1700456.
12. Ferrari E., Eeckhout E., Keller S. et al. Transfemoral versus transapical approach for transcatheter aortic valve implantation: hospital outcome and risk factor analysis. J Cardiothorac Surg. 2017;12(1):78. doi:10.1186/s13019-017-0638-9.
13. Nishimura R.A., Otto C.M., Bonow R.O. et al. 2017 AHA / ACC focused update of the 2014 AHA / ACC guideline for the management of patients with valvular heart disease: a report of the American College of Cardiology / American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2017, 70: 252–89.
14. Gargiulo G., Sannino A., Capodanno D. et al. Transcatheter aortic valve implantation versus surgical aortic valve replacement: a systematic review and meta-analysis . Ann Intern Med. 2016; 165: 334–44. https://doi.org/10.7326/M16-0060 .
15. Cao C., Ang S.C., Indraratna P. et al. Systematic review and meta-analysis of transcatheter aortic valve implantation versus surgical aortic valve replacement for severe aortic stenosis. Annals of Cardiothoracic Surgery. 2013; 2: 10–23.
16. Panchal H.B., Ladia V., Amin P. et al. A meta-analysis of mortality and major adverse cardiovascular and cerebrovascular events in patients undergoing transfemoral versus transapical transcatheter aortic valve implantation using Edwards valve for severe aortic stenosis. Am J Cardiol. 2014; 114: 1882–90. https://doi.org/10.1016/j.amjcard.2014.09.029 .

ORCID and contributionship:

Glib I. Yemets: 0000-0002-6139-6235 [A,B,D,F]
Oleksandra V. Telehuzova: 0000-0003-4801-093X [B,C,D]
Georgiy B. Mankovsky: 0000-0003-0335-7868 [A,E]
Andrii V. Maksymenko: 0000-0002-6615-9036 [E,F]
Yevhen Y. Marushko: 0000-0002-0696-9926 [E]
Arkadii A Dovgalyuk: 0000-0002-6606-394X [A,E]
Illya M. Yemets: 0000-0002-1893-0164 [A,E,F]

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CORRESPONDING AUTHOR

Oleksandra V. Telehuzova
Ukrainian Children's Cardiac Center.Clinic for Adults
24 Illienka st., 04050 Kyiv, Ukraine
tel: +380687044822
e-mail: teleguzova5@gmail.com

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ORIGINAL ARTICLE

ULTRASOUND EXAMINATION OF THE LUNGS IN THE INTENSIVE CARE UNIT IN OBSTETRIC PRACTICE

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Olena O. Karlova[1], Olena V. Kuzminska[2], Tetiana I. Trunina[2]
[1]P.L. SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KYIV, UKRAINE
[2]O.O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

The aim: Diagnosis of bronchopulmonary pathology in the intensive care unit in patients with obstetric and gynaecological diseases and to implement aspects of the BLUE protocol in an intensive care unit for pregnant women with lung diseases.

Materials and methods: The study involved pregnant women admitted to the intensive care unit at the city centre for reproductive medicine. To study the bronchopulmonary system, an Aloka 3500 ultrasound scanner was used, a chest X-ray was performed, spirometry study of the function of external respiration.

Results: Identified radiological changes in the lungs that were confirmed by ultrasound. Data on changes in external respiration indices showed the presence of respiratory failure by restrictive type and was not accompanied by a change in the gas composition of the blood (SpO_2 and $EtCO_2$).

Conclusions: technique for ultrasound examination of the bronchi-pulmonary system in pregnant women was developed and introduced in the intensive care unit. Ultrasound profiles of lung diseases were identified, which allow determining the intra and extrapulmonary changes in pregnant women in the early stages of pathology.

KEY WORDS: ultrasound examination, pregnant women, lungs, intensive care, bronchopulmonary pathology

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INTRODUCTION

Diseases of the bronchopulmonary system occupy priority positions in the structure of the morbidity of pregnant women, during this period the woman's body is most susceptible to the influence of various factors of the external and internal environment.

During pregnancy, physiological changes in the woman's bronchopulmonary system are adaptive. There are such changes as: elevation of the diaphragm by 4 cm; an increase in minute ventilation of the lungs by 30-50%, which progresses with an increase in gestational age; an increase in the minute volume of breathing from 8.4 l / min to 11.1 l / min, associated with an increase in oxygen consumption by 15-20%; dilation of the lumen of the bronchi, which leads to changes in the mechanics of respiration and form a restrictive type of respiratory failure, etc. [1, 2].

A significant effect on the course of bronchopulmonary pathology in pregnant women is exerted by the state of the immune system. During pregnancy, a "physiological tolerance" is formed, which is accompanied by a decrease in the level of T-helpers and natural killers in pregnant women, which leads to a decrease in the woman's body resistance to the altering effects of viral and bacterial agents [2].

Particular attention of doctors is given to HIV-infected women, since this is a special risk group for the development of bronchopulmonary pathology and complications associated with it. From literature data [3] it is known that, as immunodeficiency progresses in HIV-infected patients, opportunistic infections of various localizations develop (for example, tuberculosis – up to 66.5% in HIV-infected pregnant women). The occurrence of tuberculosis is possible at any stage of HIV infection and this does not depend on the level of CD^4+lymphocytes. Less common is the development of pneumocystic and cytomegalovirus pneumonia (up to 7.1%). Opportunistic infections, in most cases, cause the death of HIV-infected patients.

Damage to the lower respiratory tract, as well as their severity in patients with HIV infection, depends on the number of CD^4+lymphocytes in the blood and has a more severe course in pregnant women in the second and third trimester, which is due to the immunological state and physiological changes in the bronchopulmonary system.

Therefore, the above physiological changes in the body of pregnant women raise the question of the need for early diagnosis of extragenital pathology [4].

The inability to use X-ray methods due to its negative effect on the fetus forces researchers to develop alternative and informative diagnostic methods, such as ultrasound. A particularly urgent need for the use of these methods appeared in the intensive care unit in pregnant women with a complicated course of bronchopulmonary pathology [4, 5].

Thus, urgent ultrasound of the lungs in diagnostic accuracy is much superior to radiography performed at the patient's bed, and in sensitivity and specificity it is equal to computed tomography, which ensures the accuracy of the initial diagnosis in 90.5% of cases in patients with acute respiratory failure [6-9].

Table I. Ultrasound and radiological comparison of the clinical situation

Ultrasound and X-ray verification (confirmation)	I group N = 23		II group N = 19	
	n	M±m	n	M±m
Ultrasound and X-ray verification of pneumonia	19	45,2±7,6	18	42±5,6
Ultrasound verification and cases not confirmed radiographically	3	7,1±3,9	1	2,4±1,3
Cases that are not confirmed by ultrasound and radiological diagnostics	1	2,4±1,3	_	_

Table II. External respiration indices

Indicators of the function of external respiration	I group	II group	Control group
PEF, %	44* (37–49)	42* (40–52)	65 (62 -71)
PEF, L/sec	2,76* (2,19–3,20)	2,93* (2,59–3,27)	3,54 (3,16 - 4,20)
FVC, %	68* (63,5–74,5)	72* (68,5–77)	87 (82;92)
FVC, l	2,2*8 (2,05–2,55)	2,44* (2,16–2,69)	3.72 (3,2- 3,8)
R-R, min breathing rate	22 (17 – 25)	20 (18–23)	16 (14 -18)
SpO_2 (%)	96 (92 -97)	94 (90–97)	99 (98 – 99)
$EtCO_2$ мм рт. ст.	32 (29-34,2)	30 (30–32,5)	34 (32,2- 35,6)

Note. * - the level of significant difference in the indices of the study groups in comparison with the indices of the control group $p < 0.05$.

An ultrasound examination of the lung pathology is performed within a few minutes and contributes to the early diagnosis of bronchopulmonary diseases, and, therefore, the timely conduct of adequate therapeutic measures, which accelerates the improvement of the condition of patients [10-14].

THE AIM

The aim was to diagnosis of bronchopulmonary pathology in the intensive care unit in patients with obstetric and gynaecological diseases and to implement aspects of the BLUE protocol [15, 16] in an intensive care unit for pregnant women with lung diseases.

MATERIALS AND METHODS

The study involved 42 pregnant women admitted to the intensive care unit at the city centre for reproductive medicine (Kyiv). The main complaints at admission were: shortness of breath (78%), fever (82%), and cough(34%). The average age of the patients was 34.5 ± 1.3 years. All examined patients were pregnant women with different gestational age.

To study the bronchopulmonary system, we used an Aloka 3500 ultrasound scanner using linear (7.5 MHz) and convex sensors (3.5 MHz) in the B-M mode and colour Doppler mapping (CDC) mode.

A chest X-ray was performed in a sitting position in two projections on an X-ray unit PLX-102 Mobile X-ray.

Upon admission to the intensive care unit, all patients underwent a spirometry study of the function of external respiration using a Heaco SP 10 spirometer (China).

The data obtained are systematized and analysed using methods of mathematical statistics using the program Statistica_6, StatSoft by StatSoftInc, USA.

RESULTS

The examined patients are divided into 2 groups depending on the infectious history. The first group included 23 patients with bronchopulmonary pathology and concomitant HIV infection, hepatitis C. The second group consisted of 19 patients with bronchopulmonary pathology in the absence of an infectious history. The control group consisted of 12 women with a physiological course of pregnancy and without concomitant viral and infectious pathologies.

As a result of lung examination, the following data were obtained (Table I):

- the diagnosis of pneumonia was confirmed by signs of ultrasound (ultrasound) and by the results of x-ray examination in 19 (45.2 ± 7.6%) pregnant women;
- cases with positive signs of pneumonia by ultrasound and not confirmed radiologically in 3 women (7.14 ± 3.9%);
- cases with negative signs of ultrasound and not confirmed radiologically – 1 (2.4 ± 1.3%);

The results obtained during radiological studies in the second group showed that:

- cases with positive signs of ultrasound and X-ray examination confirmed the diagnosis of pneumonia in 18 pregnant women (42 ± 7.6%);

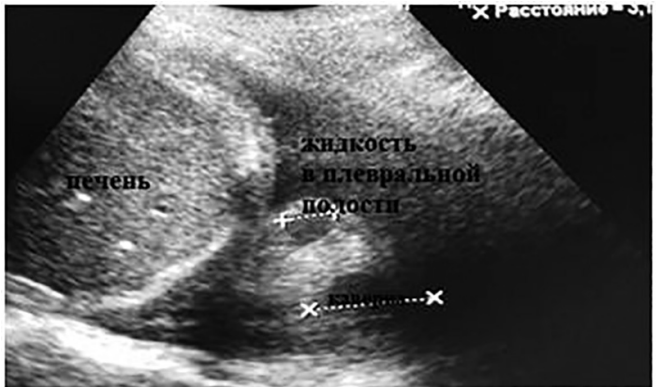

Fig. 1. Ultrasound of patient T.

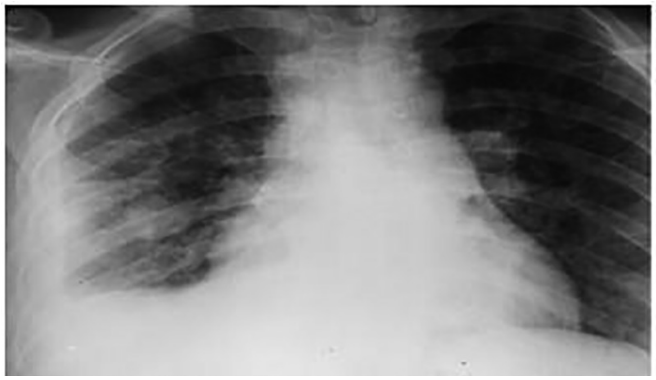

Fig. 2. Radiographic data of patient T.

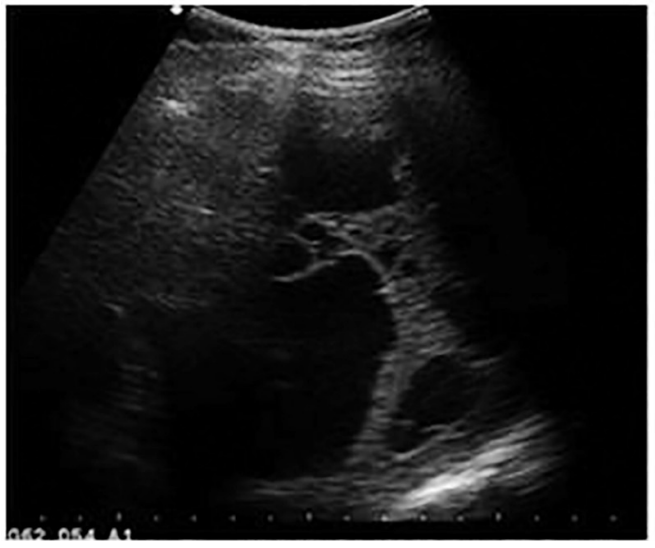

Fig. 3. Patient C.

- cases with positive signs of ultrasound and not confirmed by radiological findings were found in 1 pregnant patient (2.4 ± 1.3%).

X-ray studies were not performed in patients of the control group, because according to ultrasound, there were no signs of lung disease.

Considering the results of an X-ray examination, exudative pleurisy was most verified in patients of the first group – 21 cases (50 ± 7.7%); lung atelectasis – 6 (12.4 ± 1.3%); tuberculosis – 5 (11.9 ± 4.9%) cases. In patients of the second group, interstitial lung lesion was detected more often – 16 (84.3 ± 7.3).

Pulse oximetry was monitored for all patients and external respiration indices were evaluated (Table II). The study of the function of external respiration was carried out using a spirometer SP 10 "Naso medical technology". Using this device, we determined the forced vital capacity of the lungs (FVC) (FVC), the volume of forced expiration during the first second (FEV1) (FEV1), the peak expiratory flow rate (PEF) (PSV), the peripheral blood saturation SpO_2, the carbon dioxide content at the end expiration ($EtCO_2$). In addition, the ratio of the determined indicator to the proper indicator was evaluated.

The analysis of measurements of indicators of the function of external respiration indicates impaired ventilation of the lungs by restrictive type. There was a significant decrease in PEF compared to the control group 1.5 times in patients of group I and 1.54 times in patients of group II, respectively ($p < 0.05$). There was no significant difference in PEF between group I and group II. Patients in the study groups showed a statistically significant decrease in FVC values by 1.2-fold ($p < 0.05$) compared to the control group. These changes in indicators of external respiration indicate the presence of respiratory failure by restrictive type, which were not accompanied by changes in blood gas composition (SpO_2 and $EtCO_2$).

All patients underwent ultrasound examination of the lungs according to standard methods.

According to the ultrasound concept of the bronchopulmonary system, we examined patients with the following respiratory failure profiles: normal lung profile (23%); profile with interstitial syndrome 17%; profile with pleural effusion (60%).

Figure 1 shows the ultrasound of patient T. during pregnancy for 26 weeks with a diagnosis of admission: Tuberculosis of the respiratory system. Progressive bilateral fibrous-cavernous pulmonary tuberculosis, exudative bilateral pleurisy, respiratory failure (DN) III. Meningoencephalitis. Hyperthermic syndrome. Chronic viral hepatitis C. HIV infection.

Pregnancy 26 weeks. Diagnosis: respiratory tuberculosis. Progressive bilateral fibro-cavernous pulmonary tuberculosis, exudative bilateral pleurisy, DN III. Meningoencephalitis. Hyperthermic syndrome. Chronic viral hepatitis C. HIV infection.

Echography symptoms are represented by the presence of fluid in the pleural cavities. Against the background of exudate, single septa were found, represented by hyperechoic strands, which had an uneven thickness (4-18 mm) and sagged into the lumen of the pleural cavity.

The ultrasound data of this patient were compared with radiographic data, where cavernous lesions in the lower lobe of the right lung were verified (Fig. 2).

Pregnancy 26 weeks. The diagnosis is respiratory tuberculosis. Progressive bilateral fibrous-cavernous pulmonary tuberculosis, exudative bilateral pleurisy, DN III. Meningoencephalitis. Hyperthermic syndrome. Chronic viral hepatitis C. HIV infection.

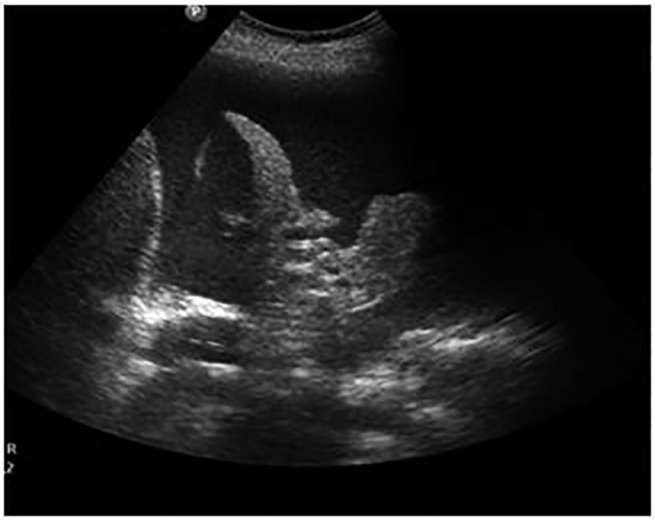

Fig. 4. Patient T.

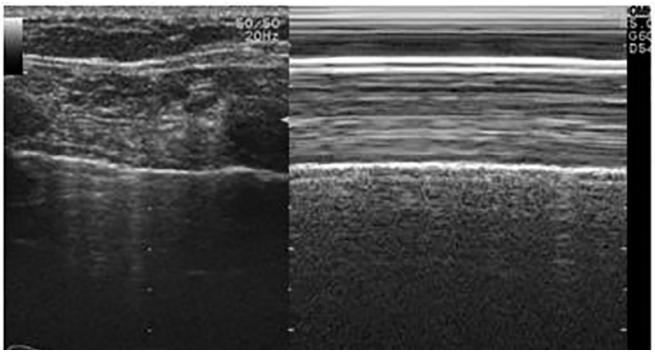

Fig. 5. Patient B.

The radiograph (Fig. 2) shows the cavity of the cavity with an annular shadow, which has, in most cases, spherical or ellipsoidal shapes. The wall thickness of the cavity is due to the fibrous capsule and perifocal inflammation.

Ultrasound examination of the lungs allows you to diagnose the minimum amount of exudate and its nature in the pleural cavities. The diagnostic value of the method is relevant in conditions where the use of an X-ray examination of the lungs is contraindicated or impossible.

Figure 3 presents the data from a study of patient C. with a pregnancy of 29 weeks and with a diagnosis of bilateral tuberculous exudative pleurisy.

The fluid in the right pleural sinus is diagnosed at the level of 2 ribs, which is an indication for pleural puncture.

The echography symptoms of pleurisy of tuberculous etiology were characterized by the following: on the background of exudate, single septa were found that were hyperechoic, had an uneven thickness (4-19 mm), and sagged into the pleural cavity. The overlays on the pleura were visualized throughout the entire visible area, or locally and were characterized by clear uneven contours, average echogenicity with a variable thickness of up to 21 mm. Their structure was characterized by homogeneity (Fig. 3).

Pregnancy 34 weeks. Bilateral exudative pleurisy.

On the background of an echo-negative fluid, reticular fibrous hyperechoic strands are visualized, the size and location of which was different for each patient and is not related to the course and duration of the disease (Fig. 3, 4).

Pregnancy 34 weeks. Exudative specific bilateral pleurisy. A valuable diagnostic aspect of ultrasound examination of the lungs is the ability to diagnose the presence and absence of a profile of pneumothorax (Fig. 5).

Pregnancy 35 weeks. Suspected pneumothorax. Bilateral lower lobar pneumonia.

On the echogram, the pleura line is visualized in the form of a hyperechoic line and the sign of a "sea breeze" in the M-mode of scanning.

DISCUSSION

The use of the ultrasound method for diagnosing pulmonary pathology in pregnant women in the intensive care unit in parallel with the X-ray method also made it possible to identify changes in the parameters of external respiration. All radiological changes in the lungs of pregnant women were confirmed by ultrasound data [5, 6].

The revealed changes in the lungs were characterized by the presence of more than 3 B lines in the corresponding lobe of the lung, which is a characteristic of the interstitial – alveolar syndrome in pneumonia. The presence of subpleural consolidations correlated with the severity of pneumonia and the presence of ventilation failure [8].

The detection of fluid in the pleural cavities had high sensitivity and specificity with a minimum volume of fluid from 15 ml, while X-ray verification of the presence of fluid in the pleural cavities was possible only with an amount of fluid with a minimum volume of 112 ml. Therefore, this ultrasound research method is the most sensitive.

In addition, it was found that ultrasound can determine intrapulmonary and extrapulmonary changes in the early stages of pathology, which is important when using this diagnostic method in pregnant women, as a gentler, fast and at the same time informative method.

Such diagnostics can be widely used in pediatric practice as a good alternative to other methods of examination.

CONCLUSIONS

1. A technique for ultrasound examination of broncho-pulmonary pathology in pregnant women has been developed and implemented.
2. Ultrasound criteria for lung diseases of pregnant women (pneumonia, pleurisy) were identified in the intensive care unit.
3. This methodology and criteria for lung diseases make it possible in the early stages of pathology to determine the intra and extrapulmonary changes in the broncho-pulmonary system in pregnant women.
4. The ultrasound picture of lung diseases is due to general clinical and ultrasonic features of the reflection of ultrasonic waves from altered structures.
5. Ultrasonic semiotics of pleurisy is characterized by the presence of echo-negative contents in the pleural cavities.

6. In patients with lung diseases, there was a restrictive type of respiratory failure without a change in the gas composition of the blood.

REFERENCES

1. Zamorina S. A., Timganova V. P., Bochkova M. S. et al. Al'fa-fetoprotein: perspektivy issledovaniya immunomoduliruyushchik svoystv [Alpha-fetoprotein: Perspectives of immunomodulation potencies' research]. Rossiyskiy immunologicheskiy zhurnal. 2016;10 (2): 236–237.(in Russian).
2. Zhang J., Tian Z. UNK cells: their role in tissue re-modelling and preeclampsia. Semin.Immunopathol. 2007;29(2): 123–133.
3. British HIV Association guidelines for the management of HIV infection in pregnant women. 2018: 114 . 2012 Updated May2014, to wydanie nie ma 114 str tylko 77. w 2018 : BHIVA guidelines for the management of HIV in pregnancy and postpartum 2018 (2020 third interim update)
4. Zanobetti M., Poggioni C., Pini R. Can chest ultrasonography replace standard chest radiography for evaluation of acute dyspnea in the ED? Chest. 2011;139(5):1140-1147.
5. Volpicelli G., Elbarbary M., Blaivas M. et al. International evidence-based recommendations for point-of-care lung ultrasound. International Liaison Committee on Lung Ultrasound (ILC-LUS) for International Consensus Conference on Lung Ultrasound (ICC-LUS.Intensive Care Med.2012;38(4):577-91.
6. Cortellaro F., Colombo S., Coen D. et al. Lung ultrasound is an accurate diagnostic tool for the diagnosis of pneumonia in the emergency department. Emerg Med J. 2012;29(1):19-23.
7. Liechtenstein D.A. Lung ultrasound in the ICU: from diagnostic instrument to respiratory monitoring tool. Minerva Anestesiol. 2012;78(11):1282-96.
8. Parlamento S., Copetti R., Di Bartolomeo S. Assessment of lung ultrasound for diagnosing pneumonia. American Journal of Emergency Medicine. 2009; 27 (4): 379-84.
9. Volpicelli G., Caramello V., Cardinale L. et al. Diagnostics of radio-occult pulmonary states by means of real-time ultrasonography of the thorax in patients with pleuritis. Ultrasound in Medicine and Biology 2008;34 (11): 1717-23.
10. Reissig A., Copetti R., Mathis G. et al. Lung ultrasound in the diagnosis and subsequent observation of the acquired community pneumonia: promising, multicentre, research of accuracy of diagnosis. Chest. 2012; 142 (4): 965 – 72.
11. Llamas-Älvarez A.M., Tenza-Lozano E.M., Latour-Perez J. The accuracy of lung ultrasonography in diagnosis of pneumonia in adults: systematic examination and meta-analysis. Chest. 2017;151(2):374-382.
12. By P.E., Morris A.M. Review: Ultrasonography of the lungs had different sensitivity and specificity of diagnosis of pneumonia in adults. Annals of Internal Medicine. 2017 J; 166 (12): JC69.
13. Galstyan G.M., Novikov V.A., Troitska V.V et al. Dlagnostika pnevmoniya z pidtrimkoyu ultrazvukovogo doslidzhennya legkih u beremennih z opuholevimi zabolonyami sistemi krovi.[Diagnosis of pneumonia with support for ultrasound examination of lungs in pregnant women with neoplastic diseases of the blood system]. Therapeutic archive. 2015; 87 (1): 79-87. (in Ukrainian).
14. Novikov V.A., Galstyan G.M., Gemdzhan E.G. et al. Ultrazvukove doslidzhennya legkih i plevralnih polostey pri pobudovi dihalnoy inedostatosti, vivilnenoyi pnevmoniyi, v onkogematologichnih boksah. [Ultrasound examination of the lungs and pleural cavities in the construction of respiratory failure, released pneumonia, in oncohematological boxes].Anesthesiology and Intensive Care. 2016; 61 (3): 183-9. (in Ukrainian).
15. Lichtenstein D.A., Mezière GA. Relevance of lung ultrasound in the diagnosis of acute respiratory failure: the BLUE protocol. Chest. 2008;134(1):117-25.
16. Liechtenstein D.A. BLUE-Protocol and FALLS-Protocol: Two applications of lung ultrasound in the severely ill. Chest. 2015;147(6):1659-1670.

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ORCID and contributionship:
Olena O. Karlova: 0000-0002-7683-9908 [A,B,D,E]
Olena V. Kuzminska: 0000-0002-8259-1611 [D,F]
Tetiana I. Trunina: 0000-0002-9870-3577 [C]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Olena V. Kuzminska
Bogomolets National Medical University
122 Mayakovsky Avenue, 8 apt., 02217 Kyiv, Ukraine
tel: +38(067) 317-34-54
e-mail:el-kuz@ukr.net

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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

DEPENDENCE OF PANCREAS FUNCTIONAL CAPACITY AT CHRONIC PANCREATITIS ON ENDOTOXICOSIS AND OTHER METABOLIC FACTORS

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Liliya S. Babinets, Olexandr S. Zemlyak, Iryna M. Halabitska, Halyna M. Sasyk, Zoreslava Ya. Onufryk
HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

ABSTRACT

The aim: Was to investigate the status of endotoxicosis parameters in patients with chronic pancreatitis depending on the presence of a combination with type 2 diabetes mellitus, as well as their impact on the functional capacity of the pancreas.

Materials and methods: 87 outpatients with CP with concomitant type 2 diabetes and without it were examined. The main group consisted of 62 patients with CP in the phase of therapeutic exacerbation in combination with diabetes mellitus in a state of sub- or full compensation, the comparison group – 25 patients with isolated CP, the control group consisted of 30 healthy individuals. The content of malonic aldehyde in the blood was determined by reaction with thiobarbituric acid, the levels of medium-molecular peptides MMP1 and MMP2 – by the method of Gabrielyan, circulating immune complexes – by precipitation in 3.75% ethylene glycol with followed photometry.

Results: The presence of active endotoxicosis and lipid peroxidation in CP was established, which was significantly more significant in the comorbidity of CP with type 2 diabetes: erythrocyte intoxication index was higher by 19.2%, the content of medium molecules MMP1 – by 29.5%, MMP2 – by 35.4%, malonic aldehyde – 10.9%, circulating immune complexes – 23.9%, ceruloplasmin – by 11.9% ($p < 0.05$)

Conclusions: A deeper level of excretory and incretory insufficiency of the pancreas in concomitant diabetes mellitus 2, which deepened with increasing endotoxicosis by the level of erythrocyte intoxication index based on an increase in the strength of significant moderate and moderate inverse correlations between it and fecal α-elastase such in isolated CP (respectively $r=-0.517$ and $r=-0.471$, $p<0.05$) and significant direct moderate and medium strength correlations between levels of HbA1c and erythrocyte intoxication index – respectively $r=0.552$ and $r=0.337$, $p<0.05$.

KEY WORDS: chronic pancreatitis, type 2 diabetes mellitus, endotoxicosis, medium molecular weight peptides, erythrocyte intoxication index, functional insufficiency of the pancreas

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INTRODUCTION

The progression of chronic pancreatitis (CP), especially in combination with type 2 diabetes mellitus (DM2), occurs under conditions of oxidative stress and chronic inflammation due to both specific and nonspecific factors that determine the constant presence of varying degrees of endotoxicosis [1, 2]. In CP, the activation of free radical or lipid peroxidation (LPO) processes leads to systemic membranopathy with the development of regenerative-plastic insufficiency syndrome at the level of the whole organism, which determines the multiorgans of the pathology with predominant lesions of barrier systems and detoxifying organs [3]. Aldehydes, LPO products, are able to activate mononuclear cells, including pancreatic star cells and Kupffer cells, which produce collagen, the liver, cause cross-linking of cytokeratins to form Mallory cells, and stimulate neutrophil chemotaxis. In CP and DM2, some of the free fatty acids coming from the intestine or formed in the liver are transported to the mitochondria, where β-oxidation and subsequent oxidation occur in the Krebs cycle. In the process of regeneration of reduced forms of coenzyme electrons, NAD and FAD are transferred to the mitochondrial respiratory chain, where phosphorylation of ADP occurs with the formation of ATP. It is known that even during normal operation of the respiratory chain, some electrons react with oxygen, which leads to the synthesis of reactive oxygen species (ROS) – superoxide anionic radicals, hydrogen peroxide, singlet oxygen, hydroxyl radical, nitroxyl radical, and others. [4]. The formation of ROS is enhanced under the influence of factors that disrupt the respiratory chain – these are endogenous and exogenous substances such as TNF-α, medium molecular weight peptides (MMP), nitric oxide, circulating immune complexes (CIC), iron, copper, ethanol, cerulop, drugs, etc. It has long been proven that long-term or excessive intake of ethanol, which is one of the etiological factors of CP, leads to the synthesis of cytochrome P450 2E1, the main enzyme of the microsomal system of ethanol oxidation, and increased ROS formation [5]. Separation of oxidation and phosphorylation leads to the formation of excess ROS, which, in turn, cause damage to mitochondrial DNA, which exacerbates dysfunction of the respiratory chain.

ROSs have direct cytotoxicity by activating Fas-mediated apoptosis of pancreatic cells. ROSs also initiate LPO, exacerbating endotoxicosis, leading to the formation of toxic compounds, including malonic aldehyde (MA), 4-hydroxynonenal F-isoprostanes, etc., which can both directly cause the death of pancreatic cells and hepatocytes, and induce cytotoxic immune reactions due to covalent binding to cellular proteins, as well as the formation of cytokeratins (Mallory cells), fibrogenesis and chemotaxis of polymorphonuclear neutrophils. In addition, ROS promote the secretion of cytokines (TNF-α, TGF-β, IL-8) by mononuclear phagocytes, which potentiate these pathological processes. It is established that the increase of LPO in CP contributes to changes in the morpho-functional properties of erythrocytes: their ability to deformation and resistance to LPO decreases, catecholamine-depositing function changes, which deepens hypoxia. Thus, the vicious circle of LPO and endotoxicosis is closed. It is proved that in CP and DM2 there is an increase in the level of CIC, which under certain conditions can be fixed in the vessels and cause an inflammatory reaction. In chronic processes, they circulate in the blood for a long time, maintaining the conditions for the defeat of target tissues [6]. It has been established that at low concentrations CICs can cause B-lymphocyte proliferation, and at high concentrations they can inhibit it. There are reports that under the influence of high concentrations of CIC there is an increase in the activity of T-suppressors, especially those that contain IgG, as well as inhibition of the activity of natural killers [7]. A well-known pro-inflammatory component is ceruloplasmin, which is, on the one hand, an active antioxidant and, on the other hand, a marker of active inflammation and endotoxicosis.

All these factors, in our opinion, can contribute to the maintenance of inflammatory chronic process in the pancreas, as well as torpid destructive changes in the tissues and organs of patients with CP in combination with DM2, deepening the functional insufficiency of the pancreas, which makes further study [8, 9]. This is important to consider in the comprehensive treatment of such comorbid patients, which would improve their quality of life, would stop the further progression of CP and diabetes through rational correction of pancreatic dysfunction [10].

THE AIM

The aim of the study was to investigate the state of the parameters of endotoxicosis in patients with chronic pancreatitis, depending on the presence of a combination with type 2 diabetes, as well as their impact on the functional capacity of the pancreas.

MATERIALS AND METHODS

We examined 87 outpatients with CP with concomitant DM2 and without it, who were registered at the Ternopil Center of Primary Health Care. The average age was (49.33 ± 11.54) years. The duration of CP in the entire cohort of patients ranged from 1-30 years, the average duration was (10.05 ± 0.83) years. The duration of comorbidity of CP and DM2 ranged from 0 to 25 years, the average duration was (8.93 ± 0.60) years, in 76% of patients the disease lasted up to 10 years, in the remaining 24% – more than 10 years, in 24% – up to 5 years. The diagnosis of CP was established according to the clinical and statistical classification proposed by the State Institution "Research Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine" in 2003, as well as the Order of the Ministry of Health of Ukraine dated 10.09.2014 № 638 "On approval and implementation of medical documents». The diagnosis of DM2 was verified by the Order of the Ministry of Health of Ukraine from № 1118 from 21.12.2012 "On approval and implementation of medical and technological documents for standardization of medical care for type 2 diabetes".

The main group consisted of 62 patients with CP in the phase of therapeutic exacerbation in combination with diabetes mellitus in a state of sub- or full compensation, the comparison group – 25 patients with isolated CP, and the study groups were comparable in age, sex, duration and treatment of the underlying disease in the previous stages. The control group consisted of 30 healthy individuals who were representative of the study groups by age, sex and social status.

The state of LPO as a marker of oxidative stress was evaluated by the content of MA, the determination of which is based on the fact that at high temperatures in an acidic environment MA reacts with thiobarbituric acid, forming a colored trimethine complex. Evaluation of endogenous intoxication (EI) was performed by the levels of MMP1 and MMP2 by the method of Gabrielyan: studied the optical density of serum at wavelengths of 254 and 280 nm, thus determining the content of those MMP, which contain aromatic amino acids, and those that do not contain. The CIC level was determined by selective precipitation in 3.75% ethylene glycol, followed by photometry. The EI index (IEI) was determined by the Togaibayev method, which is based on the idea of erythrocytes as a universal adsorbent. Determination of CP content was performed taking into account its exceptional ability to oxidize n-phenylenediamine dihydrochloride to form a violet compound.

RESULTS

In the table 1 presents the obtained data of studying the parameters of endotoxicosis in CP depending on the presence of comorbid DM2. The analysis of the obtained results allowed to prove the presence of active EI and LPO in CP, which was significantly more significant in the comorbidity of CP with DM2: erythrocyte IEI was higher by 19.2%, the content of MMP1 – by 29.5%, MMP2 – by 35.4 %, MA – 10.9%, CIC – 23.9% ($p < 0.05$). Determination of CIC content is a cost-effective marker not only of EI, but also of strengthening the protective response of the immune system by increasing the number of antigen-antibody complexes that accumulate and damage the pancreas and other organs.

Table I. Comparative analysis of the parameters of endotoxicosis and LPO at CP depending on the presence of concomitant DM2

EI parameter	Control group (n=30)	Patients with CP (n=25)	Patients with CP and DM2 (n=62)	p_1	p_2	p_3
IEI, %	27,25±1,22	49,23±2,65	58,69±1,46	<0,05	<0,05	<0,05
MMP1	334,11±2,64	438,71±21,10	568,44±18,29	<0,05	<0,05	<0,05
MMP2	147,53±1,23	206,26±10,86	279,25±7,37	<0,05	<0,05	<0,05
MA, μmol / l	2,81±0,095	5,31±0,14	5,89±0,11	<0,05	<0,05	<0,05
CIC, con. un.	64,19±1,65	104,29±1,34	129,38±1,41	<0,05	<0,05	<0,05
Ceruloplasmin, mg/l	245,60±2,61	305,47±8,91	341,70±9,45	<0,05	<0,05	<0,05

Note: 1. p_1 - the reliability of the difference in the parameters of patients with CP in relation to such control groups;
2. p_2 - the reliability of the difference in the parameters of patients with CP and DM2 in relation to such control groups;
3. p_3 - the significance of the difference in the parameters of patients with CP and DM2 in relation to such groups with isolated CP.

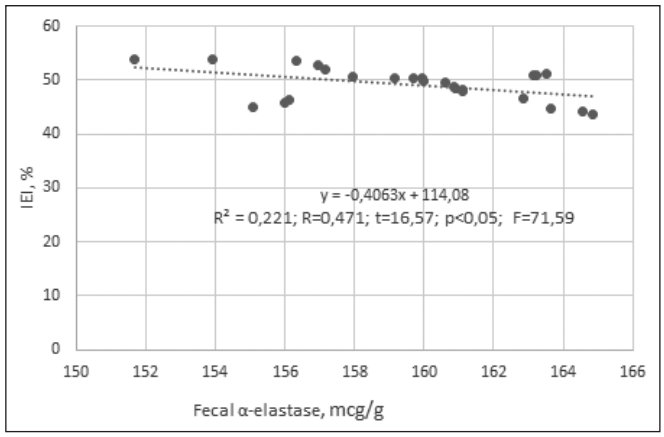

Fig. 1. Dependence of the level of fecal α-elastase on IEI,% of patients with CP

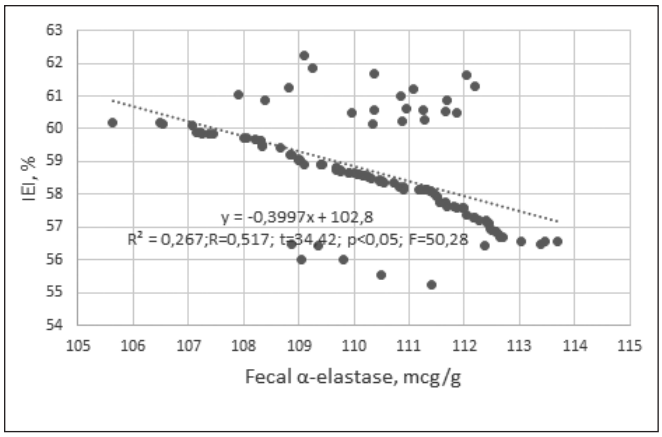

Fig. 2. Dependence of fecal α-elastase on IEI, % of patients with CP in combination with DM2

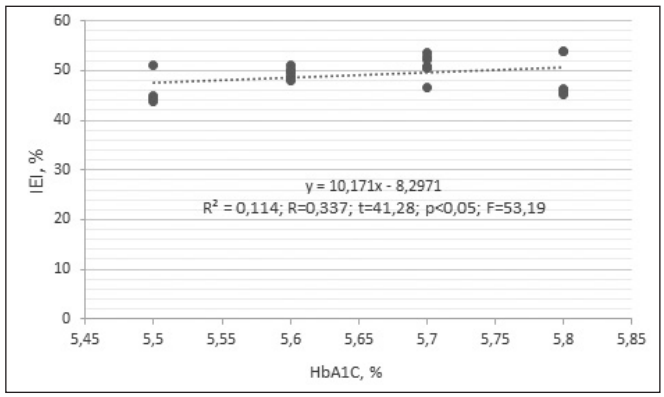

Fig. 3. Dependence of HbA1c, % on the level of IEI of patients with CP

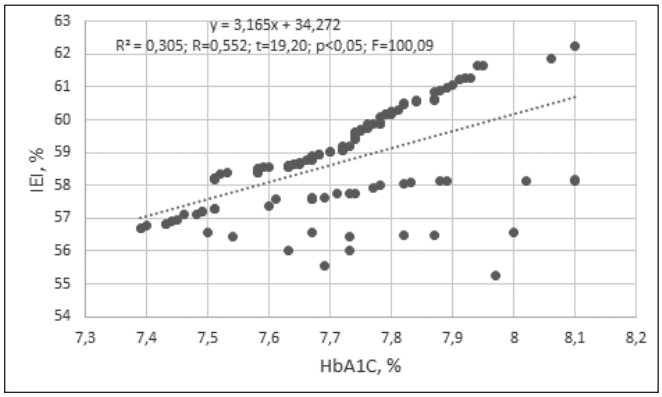

Fig. 4. Dependence of HbA1c, % on the level of IEI of patients with CP in combination with DM2

We analyzed the dependence of excretory (on the level of fecal α-elastase) and excretory (on the content of HbA1c, %) functions of the pancreas in patients with CP and CP in comorbidity with DM2 (data in Fig. 1-2) from IEI,%. IEI was considered one of the indicative integrative markers of endotoxicosis, so its relationship with the parameters of the functional capacity of the CP can be judged on the role of EI in the course of CP in combination with DM2.

The data of fig. 1-2 indicate a significant effect of IEI on the level of exocrine insufficiency of the pancreas in the content of fecal α-elastase, which was more significant in the combined course of CP and DM2 in comparison with that at CP: this is evidenced by the established moderate and moderate inverse correlations between IEI and fecal α-elastase in patients with CP and comorbidity of CP and DM2 (respectively r=-0,471 and r=-0,517, p<0,05).

According to the correlation analysis (Fig. 3-4), it was also proved to increase the strength of significant direct moderate and medium correlations between the levels of HbA1c and IEI in patients with isolated CP and CP

in combination with DM2 (respectively r=0.337 and r=0.552, p<0.05), which proved a deeper level of incretory insufficiency of the pancreas in concomitant DM2, which deepened with increasing endotoxicosis at the level of IEI.

CP activation was also found to be 11.9% (p <0.05), which can be explained by the presence of certain reserves of the antioxidant defense system even in such comorbid patients. The statistically significant increase at CP relative to the control group and especially in concomitant DM2 relative to the group of isolated CP can also be explained by the activity of inflammation and endotoxicosis in the phase of mild therapeutic exacerbation of CP, which can be performed in an outpatient setting.

DISCUSSION

Many studies have been found to investigate the effects of CP on DM2 [11, 12]. Gregory, P.K et al studied influence of non-derivative ducts of the pancreas and inflammation on the development of exocrine insufficiency of the pancreas and other trophological indicators [13]. Hollemans, R.A. et al studied the effect of changes in the pathogenetic parameters of various body systems on the development of exocrine insufficiency of the pancreas as a result of acute pancreatitis [14]. The influence of changes in the inflammatory system and other pathological changes on the development of exocrine insufficiency of the pancreas was also studied [15]. The influence of dysbiotic changes of intestinal microflora on the development and deepening of exocrine insufficiency of the pancreas has been studied [16]. The effect of exocrine insufficiency treatment on pathogenetic parameters and trophological status parameters was also studied [17]. The influence of trophological status parameters on the development of pathological processes in patients with chronic pancreatitis has been studied. [18]. However, we did not find a study that would show the influence of endotoxicosis parameters on the development of exocrine insufficiency of the pancreas and other trophological indicators.

Therefore, the obtained data proved the fact that the formation of endocrine insufficiency of the pancreas in CP in the form of DM2 occurs against the background of increased EI, LPO and immune disorders. The increase in the content of the pool of MMP, MA and CIC in the body with comorbidity of DM2 proves the presence of deepening of homeostasis due to increased catabolic processes and an increase in their toxic effects. In addition, the increase in the content of the CIC confirms the presence of an active inflammatory process in CP in the phase of therapeutic exacerbation. This figure was significantly higher in CP with concomitant DM2.

Thus, the obtained data proved the presence of inflammation, endotoxicosis and LPO in the long-term course of CP and comorbidity of CP and DM2, which is the cause and consequence of deepening exocrine and endocrine insufficiency of the pancreas according to the correlation analysis.

A limitation of our study is not too many indicators that we studied in the groups of patients during treatment and rehabilitation included in the study.

CONCLUSIONS

1) proved the presence of CP in the active course of endotoxicosis and lipid peroxidation, which was significantly more significant in the comorbidity of CP with DM2: erythrocyte intoxication index was higher by 19.2%, the content of medium molecules MMP1 – by 29.5% , MMP2 – by 35.4%, malonic aldehyde – 10.9%, circulating immune complexes – 23.9%, ceruloplasmin – by 11.9% (p<0.05);
2) proved a deeper level of excretory and incretory insufficiency of the pancreas in concomitant diabetes mellitus, which deepened with increasing endotoxicosis by erythrocyte intoxication index based on an increase in the strength of significant moderate and moderate inverse correlations between it and fecal α-elastase such in isolated CP (respectively r=-0.517 and r=-0.471, p<0.05) and significant direct moderate and medium strength correlations between levels of HbA1c and erythrocyte intoxication index – respectively r=0.552 and r=0.337, p<0 , 05.

In the future, we plan to study the manifestations of trophological insufficiency of patients with CP and DM2 and to propose methods for adequate correction of established pathogenetic and clinical disorders.

REFERENCES

1. Babinets L.S., Halabitska I.M. Chronic inflammatory process and bone tissue changes in patients with osteoarthritis and exocrine pancreatic insufficiency Lekarsky Obzor. 2020; 69 (1): 7-10.
2. Yu J.H, Kim H. Oxidative stress and inflammatory signaling in cerulein pancreatitis. World J Gastroenterol. 2014;14;20(46): 17324-9. doi: 10.3748/wjg.v20.i46.17324.
3. Babinets L., Medvid I., Herasymets I. The imbalance of the autonomic nervous system in the patients with comorbidity of hypertonic disease and chronic pancreatitis. Lekarsky Obzor. 2014; 63(6): 211-215.
4. DiMagno M.J., DiMagno E.P. Chronic pancreatitis. Curr Opin Gastroenterol. 2012;28(5):523-31. doi: 10.1097/MOG.0b013e3283567dea.
5. Kothari D., Ketwaroo G., Freedman S.D., Sheth S.G. The Impact of risk factors of chronic pancreatitis on secretin pancreatic function testing: results of a 20-year study. Pancreas. 2017;46(7):887-890. doi: 10.1097/MPA.0000000000000871.
6. Lohr J.M., Oliver M.R., Frulloni L. Synopsis of recent guidelines on pancreatic exocrine insufficiency. United European Gastroenterol J. 2013;l(2):79-83. doi: 10.1177/2050640613476500.
7. Kothari D., Ketwaroo G., Freedman S.D., Sheth S.G. The Impact of risk factors of chronic pancreatitis on secretin pancreatic function testing: results of a 20-year study. Pancreas. 2017;46(7):887-890. doi: 10.1097/MPA.0000000000000871.
8. Levy P., Dominguez-Munoz E. et al. Epidemiology of chronic pancreatitis: burden of the disease and consequences. United European Gastroenterol J. 2014;2(5):345-54. doi: 10.1177/2050640614548208.
9. Yang D., Forsmark C.E. Chronic pancreatitis. Curr Opin Gastroenterol. 2017;33(5):396-403. doi: 10.1097/MOG.0000000000000377.
10. Lew D., Afghani E., Pandol S. Chronic pancreatitis: current status and challenges for prevention and treatment. Dig Dis Sci. 2017;62(7):1702-1712. doi: 10.1007/sl0620- 017-4602-2.
11. Levy P., Dominguez-Munoz E. et al. Epidemiology of chronic pancreatitis: burden of the disease and consequences. United European Gastroenterol J. 2014;2(5):345-54.

12. Lew D., Afghani E., Pandol S. Chronic pancreatitis: current status and challenges for prevention and treatment. Dig Dis Sci. 2017;62(7):1702-1712.
13. Gregory P.C., Hoffmann K., Kamphues J., Mobeler A. The pancreatic duct ligated (Mini)pig as a model for pancreatic exocrine insufficiency in man. Pancreas. 2016;45 (9):1213-1226. doi: 10.1097/MPA.0000000000000674.
14. Hollemans R.A., Hallensleben N.D.L., Mager D.J. et al. Pancreatic exocrine insufficiency following acute pancreatitis: Systematic review and study level meta-analysis. Pancreatology. 2018;18 (3):253-262. doi: 10.1016/j.pan.2018.02.009.
15. Lindkvist B., Phillips M.E., Domínguez-Muñoz J.E. Clinical, anthropometric and laboratory nutritional markers of pancreatic exocrine insufficiency: Prevalence and diagnostic use. Pancreatology. 2015;15 (6):589-597. doi: 10.1016/j.pan.2015.07.001.
16. Manor O., Levy R., Pope C.E. et al. Metagenomic evidence for taxonomic dysbiosis and functional imbalance in the gastrointestinal tracts of children with cystic fibrosis Scientific Reports. 2016; 6, art. no. 22493. doi: 10.1038/srep22493.
17. Dominguez-Muñoz J.E. Management of pancreatic exocrine insufficiency Current Opinion in Gastroenterology. 2019;35 (5): 455-459. doi: 10.1097/MOG.0000000000000562.
18. Stigliano S., Waldthaler A., Martinez-Moneo E., et al. Vitamins D and K as Factors Associated with Osteopathy in Chronic Pancreatitis: A Prospective Multicentre Study (P-BONE Study). Clinical and Translational Gastroenterology. 2018;9 (10): 197. doi: 10.1038/s41424-018-0066-8.

Research topic: 0118U000361, Comprehensive approach to the control of symptoms, direct and distant prognosis in conditions of comorbid pathology in the clinic of internal diseases and the practice of a family medicine.

ORCID and contributionship:
Liliya S. Babinet:0000-0002-0560-1943 [A,B,C,D,E,F]
Olexandr S. Zemlyak: 0000-0002-6346-0931 [A,B,C,D,E,F]
Iryna M. Halabitska: 0000-0002-9028-7230 [A,B,C,D,E,F]
Halyna M. Sasyk: 0000-0002-9640-9155 [A,B]
Zoreslava Ya. Onufryk : 0000-0002-1273-7945 [A,B]

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CORRESPONDING AUTHOR
Liliya S. Babinets
Horbachevsky Ternopil National Medical University
14 Kupchyns'ky St., 46000 Ternopil, Ukraine
tel:+380673520743
e-mail:lilyababinets@gmail.com

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ORIGINAL ARTICLE

STUDENTS' HEALTH LEVEL AS A RESULT OF THEIR LIFESTYLE

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Grygoriy P. Griban[1], Natalia A. Lyakhova[2], Alla M. Harlinska[1], Tetiana Ye. Yavorska[1], Nataliia Ye. Kolesnyk[1], Serhii M. Hryshchuk[1], Oksana V. Obodzinska[3]

[1]ZHYTOMYR IVAN FRANKO STATE UNIVERSITY, ZHYTOMYR, UKRAINE
[2]UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY, POLTAVA, UKRAINE
[3]POLISSIA NATIONAL UNIVERSITY, ZHYTOMYR, UKRAINE

ABSTRACT

The aim: Is to study the level of knowledge, skills, abilities, and health indicators of students during the formation of their healthy lifestyle.

Materials and methods: The research was conducted at Zhytomyr Ivan Franko State University and Polissya National University in 2018-2020. Thus, 882 students (385 males and 497 females) of different specialties between the ages of 17 and 23 were examined. To assess the level of knowledge, skills, and abilities of students during the formation of a healthy lifestyle, a survey was conducted. The health level of students was assessed by the method of the qualitative express assessment of the physical health level, which made it possible to assess the dynamics of the quality of forming a healthy lifestyle.

Results: It was found that only 5.7 % of male and 6.4 % of female students had a high level of knowledge of all the health components; only 7.0 % of male and 8.3 % of female students had a high level of knowledge about the factors of health formation and skills in using them both during training and future professional activity; 20.3 % of male and 14.7 % of female students did not have knowledge about the negative impact of bad habits on health; 19.3 % of male and 18.3 % of female students did not know the components of a healthy lifestyle at all; 17.9 % of male and 21.7 % of female students did not have motivational attitudes towards a healthy lifestyle and did not know how to form them. It was found that most of the indices of both male and female students that characterize the level of students' health deteriorated significantly during their studies ($p<0.05$-0.001).

Conclusions: The research showed that most students (both males and females) did not follow a healthy lifestyle. It was reflected in the low health level of most students and the negative dynamics of the indices that characterize the level of health in the process of studying. All this requires taking into account the features of forming a healthy lifestyle of students in physical education classes at higher education institutions.

KEY WORDS: health, healthy lifestyle, physical education, students

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INTRODUCTION

The problem of forming, maintaining, and strengthening health has always been important for humans and has been the subject of medical and biological, psychological and pedagogical, and social and philosophical research [1, 2, 3]. Maintaining health, preventing diseases, and following a healthy lifestyle have always depended on the development level of the society. The formation of students' understanding and belief that health is the highest value of a person, which must be preserved and maintained continuously, requires a certain educational work during sports and health and fitness activities [4, 5].

Studying the psychological and pedagogical aspects of the formation of students' to health, scientists [6, 7] have developed criteria that are characteristics of their components, namely: the orientation of health needs, the ability to assess personal psychophysical qualities, the skills in operating specific health tools, and the creative approach to the choice of the physical activity forms. The orientation to the health values of students is formed taking into account the following psychological and pedagogical conditions: the orientation of students to the reproduction and development of valeological values; strengthening health functions in the areas of education and leisure of students; the creative attitude to the use of modern approaches in valeological and vocational education; the intensification of the development, expansion, and enrichment of the orientations of future specialists, including them in creative physical fitness and health activities [8, 9].

The scientific research [10, 11] proved that regular exercises, which is one of the main factors of a healthy lifestyle, should be taken throughout life. A special stage for a large number of people, the future elite, is studentship. Young students have always been characterized by a high level of intellectual development, the desire to acquire professional knowledge, social independence, and high motor activity. In studentship, the age formation of an organism comes to an end, motor and vegetative functions reach the highest interaction and development. The studentship is considered to be the final stage of the progressive age development of motor and mental functions. That is why in this period of biological development, the body of students is quite flexible and highly-adaptive to physical exercises. In this period of human development, physical exercises are the

Table I. The level of knowledge, skills, and abilities of the HEI students to maintain their own health (n = 385 males; n = 497 females, %)

The factors of health formation	Gender	Levels				
		High	Sufficient	Middle	Low	Poor
The components of health	males	5.7	16.6	38.7	21.8	17.2
	females	6.4	21.9	32.1	21.3	18.3
The main factors of health formation	males	7.0	17.9	39.5	24.2	11.4
	females	8.3	23.5	41.5	16.9	9.8
Bad habits and dangerous factors	males	8.1	20.5	43.9	20.3	6.7
	females	9.9	25.6	41.3	14.7	8.5
The causes of diseases and loss of working capacity	males	5.6	15.8	39.7	24.9	14.0
	females	6.2	22.9	32.3	21.1	17.5
Motor activity and its means	males	7.6	17.4	44.5	25.7	4.8
	females	7.7	24.4	33.2	18.9	15.8
Self-assessment of body state and health	males	7.8	18.7	43.4	23.4	6.7
	females	8.7	24.7	42.5	16.3	7.8

most important means of physical and mental health, the natural and biological basis for the harmonious development of personality, and effective life [12, 13].

THE AIM

The aim of this study is to investigate the level of knowledge, skills, abilities, and health indicators of students during the formation of a healthy lifestyle.

MATERIALS AND METHODS

The research was conducted at Zhytomyr Ivan Franko State University (ZSU) and Polissya National University (PNU) in 2018-2020. Thus, 882 students (385 males and 497 females) of different specialties between the ages of 17 and 23 were examined. To assess the level of knowledge, skills, and abilities of students during the formation of a healthy lifestyle, a survey and expert assessment of the students' of higher education institutions (HEI) knowledge, skills, and abilities to maintain their own health and adhere to the rules and norms of a healthy lifestyle (HLS) were conducted. The health level of students was assessed by the method of the qualitative express assessment of the physical health level (G. L. Apanasenko) [14], based on the calculated body weight, life, power, and Robinson indices and Martin-Kushelevsky test, which made it possible to assess the dynamics of the quality of forming a healthy lifestyle. The readiness of students to follow a healthy lifestyle is an integral personal formation, which is the result of purposeful training, which makes it possible to carry out personal and, in the future, professional health-improving activities. The readiness of students to implement HLS in the process of life was considered as one of the aspects of the student's readiness for personal development, and on the other hand, as the readiness for a productive life. The state of health and a healthy lifestyle were identified as the main factors determining the need for a healthy lifestyle.

The research methods included the analysis and generalization of literature sources, questionnaires, expert evaluation, testing of health indicators, methods of mathematical statistics.

RESULTS

A healthy lifestyle is a very common concept, which is interpreted by each student in their own way. Some of them consider this concept to be more physical, others – spiritual. One aspect of a healthy lifestyle is maintaining one's own body in good physical condition. It is known that the health of students is affected by many factors, and the most important of them is a lifestyle – a set of sustainable forms of life activities that determine the way of life and habits. If students constantly and persistently took care of their own health from childhood, they base the life on such stable useful habits, skills, behavior, such way of thinking, perception of others and themselves, which determine their main direction – the way to health, thus, they choose a healthy lifestyle.

A healthy lifestyle involves following certain rules that ensure harmonious development, high efficiency, spiritual balance, and health. The stable concept of "a healthy lifestyle" is based on the individual system of behavior and habits of each individual, which provides one with the necessary standard of living and healthy longevity. A healthy lifestyle involves practical actions aimed at preventing diseases, strengthening all body systems, and improving general well-being. At the same time, the conducted survey and expert assessment of knowledge, skills, and abilities of the students of higher education institutions to maintain their own health showed that only 5.7 % of male and 6.4 % of female students had a high level of knowledge of all the components of human health, 16.6 and 21.9 % respectively had a sufficient level, 38.7 and 32.1 % – the middle level, and 39.0 % of males and 39.6 % of females had a very low level or did not have such

Table II. The level of knowledge, skills, and abilities of the HEI students to comply with the norms and rules of a healthy lifestyle (n = 385 males; n = 497 females, %)

The formation factors of a healthy lifestyle	Gender	Levels				
		High	Sufficient	Middle	Low	Poor
Components of a healthy lifestyle	males	10.1	21.8	48.8	15.9	3.4
	females	9.3	20.9	51.5	13.9	4.4
Motivational and values-based attitude to a healthy lifestyle	males	9.6	22.1	50.4	11.2	6.7
	females	7.9	18.7	51.7	16.5	5.2
Psychological and pedagogical determinants of a healthy lifestyle formation	males	8.1	19.4	48.8	16.4	7.3
	females	6.8	16.9	51.8	18.3	6.2
Knowledge and skills in forming a healthy lifestyle	males	9.1	22.3	51.2	12.7	4.7
	females	9.7	19.5	54.1	12.9	3.8
Health and fitness technologies	males	7.5	16.6	43.4	25.5	7.0
	females	6.1	16.4	47.2	22.7	7.6

Table III. The dynamics of the physical health indicators of the HEI students during two years of study (n=882, X±m)

HEI	Gender	The beginning of the 2018 academic year	The end of the 2020 academic year	t	p
Body mass index, kg/m^2					
ZSU	males (n=138)	23.1±0.16	24.4±0.18	5.40	<0.001
	females (n=273)	21.9±0.10	23.0±0.12	7.04	<0.001
PNU	males (n=247)	23.3±0.12	24.2±0.14	4.88	<0.001
	females (n=224)	21.7±0.11	22.7±0.13	6.46	<0.001
Life index, ml/kg					
ZSU	males (n=138)	55.7±0.87	53.9±0.90	1.44	>0.05
	females (n=273)	45.1±0.64	42.8±0.67	2.48	<0.05
PNU	males (n=247)	55.9±0.78	54.1±0.75	1.66	>0.05
	females (n=224)	44.9±0.56	43.1±0.54	2.31	<0.05
Power index, %					
ZSU	males (n=138)	60.1±0.69	56.9±0.66	3.35	<0.001
	females (n=273)	40.5±0.48	37.4±0.51	4.43	<0.001
PNU	males (n=247)	60.3±0.52	57.7±0.49	3.64	<0.001
	females (n=224)	40.7±0.50	38.6±0.54	2.85	<0.01
Robinson index, c. u.					
ZSU	males (n=138)	94.9±0.76	95.3±0.78	0.83	>0.05
	females (n=273)	94.7±0.58	95.4±0.60	0.84	>0.05
PNU	males (n=247)	94.5±0.62	95.0±0.64	0.56	>0.05
	females (n=224)	94.2±0.52	95.0±0.55	1.06	>0.05
Martin-Kushelevsky test, s					
ZSU	males (n=138)	131.2±0.88	136.1±0.79	4.14	<0.001
	females (n=273)	137.5±0.81	142.8±0.85	4.51	<0.001
PNU	males (n=247)	129.7±0.75	133.6±0.78	3.60	<0.001
	females (n=224)	134.4±0.79	139.5±0.82	4.48	<0.001

knowledge at all (Table I). Similar results were shown by students in the research of students' knowledge about the factors of health formation, which found that only 7.0 % of males and 8.3 % of females had a high level of such knowledge, skills, and abilities and could use them both during the study and professional and life activities. At

the same time, 35.6 % of males and 26.7 % of females did not have such knowledge and did not know what factors can affect their health positively. A very large number of students did not know the impact of bad habits, dangerous factors, the causes of diseases, and the role of physical activity in their prevention. It was also found that 26.5 % of male and 33.4 % of female students could self-assess their health at a high and sufficient level and at the same time 30.1 % of males and 24.1 % of females had low and poor levels of knowledge and were unable to assess their own health.

This indicates that the existing system of physical education of students can not fully solve the problems that would change the attitude of students to their own way of life. The aim of physical education, in addition to the formation of motor skills, abilities, and physical qualities, should be educational work. The purpose of such educational work should be aimed not only at the period of studying at HEI but, above all, at students' further life and should promote the formation of positive attitudes to the factors that improve health and negative attitudes to bad habits and excess weight and obesity.

At the same time, our research showed that 19.3 % of male and 18.3 % of female students did not know the components of a healthy lifestyle at all; 17.9 % and 21.7 % respectively did not have motivational attitudes to a healthy lifestyle and did not know how to form them (Table II). Accordingly, such students do not have the need to form a healthy lifestyle, they lack the knowledge and skills how to do it or appropriate physical culture and health technologies to improve their own health.

In order to check the effectiveness of students' compliance with a healthy lifestyle and assess their health, the method of G. L. Apanasenko was used. Thus, the analysis of the body mass index showed that its dynamics had a negative trend: the body mass index of both male and female students deteriorated significantly during the 1st – 2nd years of study ($p<0.001$) (Table III). It indicated the low efficiency of health and fitness activities. In particular, classes were held only in the second year once a week at ZSU in 2018-2019, and only in the first year for one term in the 2019-2020 academic year. In terms of PNU, classes were held in the 1st – 2nd years of study for two years. Therefore, the difference between the indicators of body mass index at the beginning and end of the study of the PNU students was smaller, in comparison to ZSU students.

The study of the life index showed that the ZSU students had a greater difference between the indicators at the beginning and end of the study and, consequently, a worse level of respiratory function. It is important to note that female students of both HEI were recorded to have more pronounced negative changes in the life index than male students: the difference between the initial and final data of the male students of both HEI was unreliable ($p>0.05$), and that of female students – significant ($p<0.05$).

The analysis of the power index confirmed the low efficiency of health and fitness activities at HEI, especially at ZSU, where the number of physical education classes was lower. The power capabilities of the students (both men and women) of both HEI at the beginning of the study was assessed as lower than the middle, and at the end of the study – as low. The analysis of the Robinson index showed that it tended to deteriorate concerning the students (both men and women) of both HEI, but no significant difference between student performance at the beginning and end of the study was found ($p>0.05$).

The study of the results of the Martin-Kushelevsky test (heart rate recovery after 20 squats in 30 s) showed a significant ($p<0.001$) difference between the indicators before and after the study; the indicators of the heart rate recovery duration of the students of all groups were found to be significantly lower than they were at the beginning, which indicated a deterioration in the effectiveness of students' recovery processes. It proved the lack of effectiveness of the modern physical education process at HEI in terms of promoting the health of students and the formation of their healthy lifestyle.

DISCUSSION

Scientists have different interpretations of health. Some scientists [2, 3, 15] present health as a state with constantly changing environmental conditions in relation to the adaptive capacity of the human body. Other scientists [4, 7] consider health as a set of abilities of an individual and the features of one's behavior, which can be used to determine the liability to diseases, while health is the sum of the reserve capabilities of the main functional systems of the human body. At the same time, every year the health status of young people deteriorates by an average of 10-15 %, the level of physical and mental health decreases, the socio-cultural values of physical education are leveled [1, 13]. The deterioration of students' health is also associated with detraining, stress, negative environmental impact, failure to perform ergonomics, the lack of physical activity, resulting in an increase in the number of students with disabilities [5, 9, 11].

The study of the compliance with and formation of a healthy lifestyle by students has many different interpretations and recommendations. It indicates that there is no single way to maintain a healthy lifestyle and active life. To form an attitude to a healthy lifestyle and its observance by students both during their studies at HEI and in later life, it is necessary to begin with the assessment of the availability of knowledge and skills in following a healthy lifestyle; the assessment of health status, the presence of bad habits, diseases, and disorders of the musculoskeletal system and the ways to eliminate them; the awareness of each student of the need to maintain a healthy lifestyle, showing interest in specific types of its manifestation; the assessment of students' activity in terms of meeting the requirements of a healthy lifestyle; the implementation of health measures in life; the formation of a positive attitude of students to the means of physical culture and sports and the discipline of Physical Education; the formation

of a psychological attitude to maintaining high motor activity, a negative attitude towards bad habits, excess weight, etc.; the identification of the types of physical activity that meet the individual capabilities of students and their temperaments best; building confidence that the time spent exercising is compensated by good health; goal setting and compliance with it, despite fatigue, the lack of time, bad weather, etc.; the availability of different options for individual programs of physical culture and health-improving classes.

CONCLUSIONS

1. The modern system of physical education at HEI should provide training of students in terms of the formation of a healthy lifestyle by educating conscious responsibility for their own health, personality-oriented choice of goals, means, and methods of its correction taking into account their own needs and motivation, promoting health, free and conscious choice of physical culture and health technologies of training and the awareness of one's own responsibility for the realization of this choice.
2. The process of teaching students to form a healthy lifestyle is considered as a special combination of internal factors of physical culture: morphofunctional, psychological, physical, and personal developments and external factors characteristic of modern conditions of physical education and optimization of the physical education at HEI in the direction of preservation, strengthening, and formation of the health of students.
3. Psychological and pedagogical conditions for the formation of students' healthy lifestyle are the creation of a healthy educational environment as a space of individual opportunities to choose the physical activity for physical development, physical fitness, health promotion, and the effective forms of physical culture and health-promoting activities; the formation of a conscious motivational and values-based attitude of students to participate in sports and health and fitness activities and a stable need for physical self-improvement; taking into account the age and individual characteristics of students, improving the pedagogical competence of physical education teachers as a factor in mastering health-preserving learning technologies.

REFERENCES

1. Mozolev O., Bloshchynsky I., Alieksieiev O. et al. Influence of modern fitness technologies on the state of health and development of motor abilities of 17–19-year-old female students. Journal of Physical Education and Sport. 2019; 19(3): 917-924. doi:10.7752/jpes.2019.s3132.
2. Prontenko K., Griban G., Aloshyna A. et al. The physical development and functional state as the important components of the students' health. Wiad. Lek. 2019; 72(12): 2348-2353. doi: 10.36740/WLek201912115.
3. Gruzieva T., Galiienko L., Pelo I. et al. Health and lifestyle of students' youth: status, problems and ways of solution. Wiad Lek. 2018; 71(9): 1753-1758.
4. Prontenko K., Griban G., Dovgan N. et al. Students' health and its interrelation with physical fitness level. Sport Mont. 2019; 17(3): 41-46. doi 10.26773/smj.191018.
4. Griban G., Lyakhova N., Tymoshenko O. Current state of students' health and its improvement in the process of physical education. Wiad. Lek. 2020; 73(7): 1438-1447. doi: 10.36740/WLek202007124.
5. Zhamardiy V., Shkola O., Okhrimenko I. et al. Checking of the methodical system efficiency of fitness technologies application in students' physical education. Wiad Lek. 2020; 73 (2): 332-341. doi: 10.36740/WLek202002125.
6. Griban G., Kobernyk O., Terentieva N. et al. Formation of health and fitness competencies of students in the process of physical education. Sport Mont. 2020; 18(3): 73-78. doi: 10.26773/smj.201008.
7. Prysiazhniuk S., Tolubko V., Oleniev D. et al. The influence of physical activities on biological age parameters of the first-year female students from the special medical department. Journal of Physical Education and Sport. 2018; 18(2): 561-564. doi:10.7752/jpes.2018.02081.
8. Prontenko K., Griban G., Prontenko V. et al. Health improvement of cadets from higher military educational institutions during kettlebell lifting activities. Journal of Physical Education and Sport. 2018; 18(1): 298-303. doi: 10.7752/jpes.2018.01040.
9. Griban G., Prontenko K., Zhamardiy V. et al. Professional stages of a physical education teacher as determined using fitness technologies. Journal of Physical Education and Sport. 2018; 18(2): 565-569. doi:10.7752/jpes.2018.02082.
10. Prontenko K., Bloshchynskyi I., Griban, G. et al. Formation of readiness of future physical culture teachers for professional activity. Universal Journal of Educational Research. 2019; 7(9): 1860-1868. doi: 10.13189/ujer.2019.070903.
11. Griban G., Yavorska T., Tkachenko P. et al. Motor activity as the basis of a healthy lifestyle of student youth. Wiad. Lek. 2020; 73(6): 1199-1206. doi: 10.36740/WLek202006123.
12. Prontenko K., Griban G., Bloshchynskyi I. et al. Improvement of students' morpho-functional development and health in the process of sport-oriented physical education. Wiad Lek. 2020; 73(1): 161-168. doi: 10.36740/WLek202001131.
13. Griban G., Tymoshenko O., Arefiev V. et al. (2020). The role of physical education in improving the health status of students of special medical groups. Wiad. Lek. 2020; 73 (3): 534-540. doi: 10.36740/WLek202003125.
14. Apanasenko G. L. Knygha o zdorovj'e [Health Book]. Kyev: Medknygha; 2007: 132 p. (In Russian).
15. Mozolev O., Bloshchynskyi I., Prontenko K. et al. Influence of fitness techniques integration on the development of physical qualities and morpho-functional state of adult females. Human Movement. 2021; 22(1): 57-65. https://doi.org/10.5114/hm.2021.98465.

ORCID and contributionship:

Grygoriy P. Griban: 0000-0002-9049-1485 [A,F]
Natalia A. Lyakhova: 0000-0003-0503-9935 [E,F]
Alla M. Harlinska: 0000-0001-7859-8637 [B,D]
Tetiana Ye. Yavorska: 0000-0001-6104-2202 [C,F]
Nataliia Ye. Kolesnyk: 0000-0001-9384-9369 [A,E]
Serhii M. Hryshchuk: 0000-0002-5553-8110 [D,E]
Oksana V. Obodzinska: 0000-0002-4832-0826 [B,C]

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Grygoriy P. Griban
Zhytomyr Ivan Franko State University
40 Velyka Berdychivska St., 10002 Zhytomyr, Ukraine
tel: +380973341092
e- mail: gribang@ukr.net

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ORIGINAL ARTICLE

EPIDEMIOLOGY OF THE PREVALENCE OF PHENOTOPIC SIGNS UNDIFFERENTIATED CONNECTIVE TISSUE DYSPLASIA SYNDROME IN WOMAN WITH MISCARRIAGES

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Tunzala V. Ibadova, Volodymyr V. Maliar, Vitalii V. Maliar, Vasyl V. Maliar
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: Study of the relationship between cervical insufficiency and dysplastic stigma in miscarriages.

Materials and methods: 80 pregnant women were examined at 23-27 weeks of gestation.) Group I included 40 pregnant women with the threat of premature birth, with habitual miscarriage and correction of cervical insufficiency (CI) by using pessary in the anamnesis. Group II consisted of 20 pregnant women with the threat of premature birth and correction of CI by using pessary without the burden of habitual miscarriage, the control group of 20 almost healthy pregnant women.

Results: The studies revealed phenotypic signs of dysplastic stigmatization in 39 (97.5 + 2.5%) pregnant women of group I, in 18 (90.0 + 6.9%) group II and in 4 (20.0 + 9.2%) control, which indicates a high prevalence of connective tissue dysplasia in women with CI, which also has a laboratory reflection in the form of increased excretion per day of oxyproline and a decrease in total glycosaminoglycans in both groups at risk of preterm birth.

Conclusions: The most common gestational complication in women with connective tissue dysplasia is the risk of premature birth. Improving existing and finding new diagnostic and therapeutic measures for women with UDCTD will reduce the risk of preterm birth.

KEY WORDS: UDCTD, preterm birth, pregnancy, glycosaminoglycans, oxyproline, dysplastic stigmatization

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INTRODUCTION

The urgency of the problem of miscarriage in women with undifferentiated forms of connective tissue dysplasia is primarily due to the high prevalence that occurs in the general population up to 80%[1]. Miscarriages is the most common complication of the gestational process. Babies born at 23-27 weeks of gestation cause up to 50% perinatal mortality.

One of the significant factors in the genesis of prematurity is the syndrome of undifferentiated connective tissue dysplasia (UDCTD), which in women with cervical insufficiency (CI) of unknown origin is about 30%, and the frequency of premature birth (PB) with CI correction reaches 30 % [2,3,4]. The main causes of PB in this case are rupture of membranes, chorioamnionitis, eruption of the suture on the cervix. It is known that in the human body connective tissue performs a structural, retaining, supporting, mechanical, homeostatic and immunological function[5,6,7].

Abnormal connective tissue structure in women with systemic tissue dysplasia is closely associated with disruption of both collagen synthesis and metabolism and changes in biodegradation. At defects of structure of collagen, elastin, glycopeptides, proteoglycans, fibroblasts there can be an inferiority of a connective tissue stroma of a uterus, and in particular an internal os of the cervix, leading to the CI.

Congenital defects of systemic connective tissue dysplasia are manifested by various stigmatization. Differentiated forms have pronounced genetic markers, undifferentiated forms are manifested by phenotopic markers.

Recently, there is no objection to progressive growth in populations of individuals with dysplastic phenotypes, which is considered as a result of anthropogenic factors [8, 9].

It is undeniable that undifferentiated connective tissue dysplasia plays an important role in asymptomatic shortening and dilation of the internal os of the cervix, which contributes to premature birth [9].

Timely detection of dysplastic stigma will improve the diagnosis and prediction of miscarriages on the background of CI, which is of great scientific and practical importance.

THE AIM

Study of the relationship between cervical insufficiency and dysplastic stigma in miscarriages.

MATERIALS AND METHODS

80 pregnant women were examined at 23-27 weeks of gestation. Group I included 40 pregnant women with the threat of premature birth, with recurrent spontaneous

Table 1. (Frequency of phenotypic dysplastic stigmatization of research groups) (M±m)

Phenotypic signs	Groups of pregnant women		
	I group (n=40)	II group (n=20)	Control (n=20)
Ectodermal dysplastic stigmatization			
subcutaneous venous plexus	27,5±7,3	15,0±8,2	5,0±2,5
blue sclera	27,5±7,3	15,0±8,2	–
Visual impairment	22,5±7,9	15,0±8,2	–
skin depigmentation	22,5±7,9	10,0±6,9	5,0±2,5
Hyperplasticity of the skin	22,5±7,9	15,0±8,2	–
pale skin	12,5±5,3	10,0±6,9	–
Hemangioma or telangiectasia	7,5±4,2	5,0±2,5	–
Bone and skeletal dysplastic stigmatization			
hypermobility of the joints	72,5±7,1	55,0±11,4	20,0±9,2
posture disorders	57,5±7,9	55,0±11,4	20,0±9,2
asthenic body structure	52,5±7,2	45,0±10,4	–
sandal-shaped slit on the foot	42,5±7,6	10,0±6,9	–
abnormalities in tooth growth	15,0±5,7	10,0±6,9	–
Visceral dysplastic stigmatization			
renal dysplasia	77,5±6,9	50,0±11,5	5,0±3,2
abnormal forms of the gallbladder	65,0±7,6	50,0±11,5	5,0±3,2
mitral valve prolapse	52,5±7,9	45,0±6,4	10,6±4,9
open oval hole	17,5±6,1	10,0±4,9	2,0±1,2
abnormal structure of the uterus	7,5±3,4	5,0±2,2	–
umbilical hernia	5,0±2,2	–	–

miscarriage and correction of cervical insufficiency (CI) by using pessary in the anamnesis. Group II consisted of 20 pregnant women with the threat of premature birth and correction of CI by using pessary without the burden of recurrent spontaneous miscarriage, the control group of 20 almost healthy pregnant women.To study the condition of the connective tissue, which included a clinical evaluation of dysplastic stigmatization, the total level of glycosaminoglycans in the serum and excretion of oxyproline in the urine were determined.

Statistical processing of the obtained data was performed using the programs "Microsoft Excel. When using medical and statistical analysis, the mean values and error (M + m) were calculated, and the probability of the difference between the groups was determined by the Student's t-test.) All results were considered valid if $p < 0.01$ for the survey results and $p < 0.05$ in all other cases.

RESULTS AND DISCUSSION

The studies revealed phenotypic signs of dysplastic stigmatization (DS) in 39 (97.5 ± 2.5%) pregnant women of group I, in 18 (90.0 ± 6.9%) group II and in 4 (20.0 ± 9.2%) control. Ectodermal dysplastic stigmas were represented by subcutaneous venous plexus, blue sclera, visual disturbances, depigmentation, pallor, skin hyperplasticity, hemangiomas or telangiectasia. and stretch marks.

Among the skeletal stigmas should be noted asthenic body structure, posture disorders, hypermobility of the joints, sandal-shaped slit on the foot, abnormalities in tooth growth. Visceral phenotypic signs of dysplastic stigmatization are represented by renal dysplasia, abnormal forms of the gallbladder, mitral valve prolapse, open oval hole (d = 2.8 ± 1.3 mm), abnormal structure of the uterus, umbilical hernia.

The threshold of phenotypic stigmatization is significantly higher in groups I and II ($p < 0.05$) than the control.

Among pregnant women of groups I and II there was an increased excretion per day of oxyproline, which amounted to 60.2 ± 31.3 and 57.0 ± 23.1 mg / day, respectively, against the background of reduced total serum glycosaminoglycans, which did not differ significantly in groups.

The results of studies revealed among pregnant women at risk of preterm birth in cervical insufficiency a high frequency of dysplastic stigmatization, which may indicate molecular genetic mechanisms of development, collagenopathy in this obstetric pathology. According to modern ideas, the signs of undifferentiated connective tissue dysplasia(UDCTD) are considered as a condition caused by inhibition of the activity of enzymes

of various parts of the mitochondrial, the essence of which is reduced to "mitochondrial dysfunction"[10].

It should be noted that the etiopathogenesis of the premature birth in women with cervical insufficiency on the background of UDCTD for which there are a number of reasons, namely: genetic predisposition to collagenopathy, metabolic changes, chronic inflammation of the female genitals, micronutrient deficiency[11,12,13].

According to our data in the I group of pregnant women the asthenic type of the constitution coinciding with data of other authors prevailed.[14,15]. Among other manifestations of bone pathology, the most common were joint hypermobility, posture disorders and abnormalities in tooth growth, which can be closely related to both disorders of phosphorus-calcium metabolism and magnesium deficiency[16,17,18].

Diagnostically significant markers of visceral manifestations of dysplasia were found: renal dysplasia, abnormal forms of the gallbladder, mitral valve prolapse, open oval hole, abnormal structure of the uterus. Along with visceral markers of dysplasia, ectodermal stigmas in the form of varicose veins, increased skin extensibility, depigmentation, the appearance of stretch marks and umbilical hernia during pregnancy were quite common in these patients. Among patients of groups I and II there was an increased excretion per day of oxyproline, which was 60.2 ± 31.3 and 57.0 ± 23.1 mg / day, respectively, against the background of reduced total levels of serum glycosaminoglycans, which did not differ significantly in groups, but significantly higher than the control group.

CONCLUSIONS

1. The most common gestational complication in women with UDCTD is the premature birth on the background of cervical insufficiency.
2. Improving existing and finding new diagnostic and therapeutic measures in pregnant women with UDCTD, will significantly reduce the risk of premature birth.

REFERENCES

1. Nechaeva G., Viktorova I., Druk I. Displaziya soedinitel`noj tkani: rasprostranennost`, fenotipicheskie priznaki, assocziaczii s drugimi zabolevaniyami [Connective tissue dysplasia: prevalence, phenotypic features, associations with other diseases].Doctor.2006;1:19-23.(in Russian).
2. Holianovskyi O.V. Mekhedko V.V. Halych I.D. Bachynska M.A. Istmiko-tservikalna nedostatnist i suchasni metody korektsii [Cervical insufficiency:Modern correction methods].Ukranian medical journal "Female doctor".2012;2:39-45. (in Ukrainian).
3. Vink J., Feltovich H. Cervical etiology of spontaneous preterm birth. Semin Fetal Neonatal Med. 2016; 21(2): 106–112. DOI: 10.1016/j.siny.2015.12.009.
4. Lukina T., Pavlov O., Kozina E. Management of pregnant women with undifferentiated connective tissue dysplasia. Journal of New Medical Technologies eJournal. 2015; 9(4). DOI: 10.12737/16165.
5. Dietz H. P., Steensma A. B. Which women are most affected by delivery related changes in pelvic organ mobility?: Eur J Obstet Gynecol Reprod Biol. 2003 Nov 10;111(1):15-8. doi: 10.1016/s0301-2115(03)00155-6.
6. Lukianenko N.S. Mistse nedyferentsiiovanoi dysplazii spoluchnoi tkanyny v patolohii dytiachoho viku [The place of undifferentiated connective tissue dysplasia in the pathology of childhood]. Child Health.2015;2(61):80-85. (in Ukrainian).
7. Shcherbyna M.O. Markery rozpadu kolahenu v rozvytku prolapsu henitalii u zhinok v perymenopauzi [Markers of collagen breakdown in the development of genital prolapse in perimenopausal women]. Current issues of pediatrics, obstetrics and gynecology.2016;2:111-114. (in Ukrainian).
8. Gurbanova S.R. Rol nedifferenczirovannoj displazii soedinitel`noj tkani v patogeneze istmiko-czervikal`noj nedostatochnosti [The role of undifferentiated connective tissue dysplasia in the pathogenesis of cervical insufficiency]. Materials of the 14th Congress of Obstetricians and Gynecologists of Russia.2008;63-64.(in Russian).
9. Prokip U.Ye. Udoskonalennia diahnostychno-terapevtychnykh zakhodiv pry idiopatychnii istmiko-tservikalnii nedostatnosti [Improvement of diagnostic and therapeutic measures in idiopathic cervical insufficiency]. 2016: 20 p. (in Ukrainian).
10. Torshin I.Yu., Gromova O.A. Molekulyarny`e mekhanizmy` vozdejstviya magniya na displaziyu soedinitel`noj tkani [Connective tissue dysplasia, cell biology and molecular mechanisms of magnesium action]. Russian medical journal.2008; 16(4):230-238.(in Russian).
11. Abrahamovych O. O. Faiura O. P. Abrahamovych U. O. Komorbidnist: suchasnyi pohliad na problemu; klacyfikatsiia (povidomlennia pershe) [Comorbidity: a modern view of the problem; classification (first message)].Lviv Clinical Bulletin. 2015; 4 (12): 56-64. (in Ukrainian).
12. Magomedov S., Zhabchenko I., Oleshko V. et al. Features of connective tissue metabolism and microelements in blood serum of pregnant women with cervical insufficiency.eureka:healthsciences. 2018;6:30-36. DOI:http://eu-jr.eu/health/article/view/784.
13. Nikitin V. A., Vasil`eva L. V., Tolsty`kh E. M. et al. Rol sistemnogo vospaleniya v razvitii komorbidnosti pri khronicheskoj obstruktivnoj bolezni legkikh [The role of systemic inflammation in the development of comorbidity in chronic obstructive pulmonary disease]. Tuberculosis and lung disease journal. 2017;6(95):61-65(in Russian).
14. Shibel'gut N.M., Zakharov I.S., Mozes V.G. Techenie beremennosti i iskhody` rodov u zhenshhin s nedifferenczirovanny`mi formami displazii soedinitel`noj tkani[Clinical implications of phenotypic and biochemical manifestations of undifferentiated forms of connective tissue dysplasia in pregnant women].Medicine in Kuzbass. 2009;8(6):28-32 (in Russian).
15. Smirnova T.L., Gerasimova L.I. Features of clinical manifestations of the syndrome of undifferentiated connective tissue dysplasia. Doctor.Ru. 2018;8(152):40-44. DOI: 10.31550/1727-2378-2018-152-8-40-44.
16. Smirnova T.L., Gerasimova L.I., Sidorov A.E. et al. Osobennosti techeniya beremennosti i rodov u zhenshhin s sindromom nedifferenczirovannoj displazii soedinitel`noj tkani [Features of the course of pregnancy and childbirth in women with the syndrome of undifferentiated connective tissue dysplasia]. Practical medicine. 2018;16(6):39-44. (in Russian).
17. Domnitskaia T.M., D'iachenko A.V., Kupriianova O.O. et al. Klinicheskoe znachenie primeneniya magniya orotata u podrostkov s sindromom displazii soedinitel`noj tkani serdcza [Clinical value of the use of magnesium orotate in adolescents with syndrome of cardiac connective tissue dysplasia]. Cardiology. 2005; 3(45):76-81. (in Ukrainian).
18. Shvarts H. Ya. Vitamin D, D-gormon i al`fakal`czidiol: mediczinskie, molekulyarno-biologicheskie i farmakologicheskie aspekty [Vitamin D, D-hormone and alfacalcidol: medical, molecular biological and pharmacological aspects].Ukrainian rheumatological magazine. 2009;3:63-69. (in Ukrainian).

ORCID and contributionship:
Tunzala V. Ibadova : 0000-0003-0113-8995 [A,C]
Vasyl V. Maliar: 0000-0001-9950-5014 [B,D,F]
Vitalii V. Maliar: 0000-0002-1310-535X [E,F]
Volodymyr V. Maliar: 0000-0003-0350-3255 [D,F]

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CORRESPONDING AUTHOR
Tunzala V. Ibadova
Uzhhorod national University
1 Narodna sqr., 88000 Uzhhorod, Ukraine
tel: +380506303138
e-mail: tunzala.ibadova@uzhnu.edu.ua

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ORIGINAL ARTICLE

THE STUDY OF ACTION THE OZONE IN THE SURGICAL TREATMENT OF INFLAMMATORY PROCESSES BY DIABETES MELLITUS

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Svitlana Y. Karatieieva, Nataliia Y. Muzyka, Karolina I. Yakovets, Oksana V. Bakun, Ksenia V. Slobodian
BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

The aim: was to improve the course of purulent wounds in diabetes by using physical therapies.

Materials and methods: we investigated 122 patients with diabetes mellitus and wound processes. We divided all patients on the two groups. Our study groups were 50 patients who had therapy by ozone together with other general treatment and surgery treatment (daily dressings, ointments and solutions). Our control groups were 72 patients, who had treated only by general methods and surgery treatment (daily dressings, ointments and solutions).The patients of our explore group had received intravenous injection of saline ozone solution together with other general treatment and surgery treatment (daily dressings, ointments and solutions).

Results: it had studied of indicators of oxidative modification of proteins, lipid peroxidation in blood plasma, antioxidant protection during this period, there were no significant changes in these parameters between the control and experimental groups. As the result of the study was found that the leukocyte index of intoxication in patients in the main and control groups was almost indistinguishable. Regarding the indexes of hematological index of intoxication, which decreased during the treatment of patients of the main group, and different from the patients of the control group, in which this indicator increased sharply. The result of the study of changes in the indicators of sorption capacity of erythrocytes showed that the level of sorption capacity of erythrocytes in patients in the main group during treatment, also decreased, compared with patients in the control group.

Conclusions: Therefore, the use of ozone in the treatment of patients with complicated forms of diabetes does not cause a negative impact on the dynamics of homeostasis and indicators of the level of intoxication of the body. Ozone therapy has a beneficial effect on the course and wound process in patients with diabetes with purulent processes.

KEY WORDS: diabetes mellitus, wounds, physical methods, indices of homeostasis

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INTRODUCTION

Diabetes is a disease that affects more than 425 million people in the worldwide. There are nearly 1.3 million patients in Ukraine, and it are increasing every year. Almost 104,000 Ukrainians have been diagnosed with diabetes for the first time in 2017 – 2% more than in 2016. [1, 2]

During from ten to fifteen years, diabetes can be cause a great deal of damage to the whole body, especially if there are significant complications, even if all the requirements for treatment and diet are met. Most often, with this disease, the composition of the blood changes, which leads to chronic damage to all vital organs. The peripheral vessels, especially the lower extremities, suffer first of all, which leads to the occurrence of «diabetic foot» syndrome. In the treatment of this complication, it is important to use, along with drugs that adjust blood sugar and symptomatic treatment, as well as local treatment methods.

The treating diabetes and its complications is an extraordinary task. In this regard, it is important to study in detail the causes and course and various methods of treatment of diabetes, which makes it possible to detect the presence of certain activity of physical methods of treatment of this pathology. [3, 4].

Diabetic foot – is defeat of the feet of patients with diabetes in the form of purulent-necrotic processes, ulcers and bone and joint lesions, occurs against changes in the peripheral nerves, blood vessels, skin and soft tissues, bones and joints. It is a major cause of lower extremity amputations in patients with diabetes mellitus. There are vascular, nerve-trophic and mixed forms of diabetic foot. [5, 6, 7].

The main processes in progression of inflammatory complications of wounds in ill by diabetes mellitus are exactly the changes regarding of lipid peroxidation, antioxidant protection and the oxidative modification of proteins [8, 9].

Therefore, the challenge is to find new ways to treat the complications of diabetes. It is also important to take into account the maximum effectiveness and low cost of treatment. Exactly such methods of treatment include physical methods.

The finding effective and inexpensive therapies is one of the most important medical searches. And given that the number of patients with diabetes is increasing dramatically, so research into this area is extremely important to treat the complications of diabetes [10].

In the treatment of physical methods also use ozone therapy, because ozone has different properties, in partic-

ular has a pronounced antibacterial effect, hematopoiesis, improves reparative processes and more. [11].

THE AIM

The aim of our work was to improve the course of purulent wounds in diabetes by using physical therapies.

MATERIALS AND METHODS

In our work, we had investigated 122 patients with diabetes mellitus and the wound processes. We had divided all patients on the two groups.

The our study groups were 50 patients who had therapy by ozone together with other general treatment and surgery treatment (daily dressings, ointments and solutions).

The our control groups were 72 patients, who had treated only by general methods and surgery treatment (daily dressings, ointments and solutions).

The patients of our explore group had received intravenous injection of saline ozone solution together with other general treatment and surgery treatment (daily dressings, ointments and solutions).

The patients our study groups had received ozone solution for 7-12 days.

The our control groups had had treated only by general methods and surgery treatment (daily dressings, ointments and solutions).

The presence of inflammatory processes was evaluated by the patient's admission to the hospital, at the time of receiving appropriate treatment, and at the time of recovery.

We had studied the levels of oxidative modification of proteins in blood plasma and the indicators of lipid peroxidation in blood plasma had studied too.

Also, to evaluate the effect of ozone therapy on the course of the wound process and to evaluate the effectiveness of this physical method of treatment in patients with purulent wounds in diabetes, we had evaluated the leukocyte index of intoxication. Were leukocyte index of intoxication was normal about 1,0±0,5.

RESULTS

During the course of observation in the treatment of patients with complicated forms of diabetes mellitus, which in addition to conservative treatment, various surgical interventions were performed (resections and disarticulations of the phalanges of the fingers of the lower extremities, opening of the suppurative processes of the anterior abdominal wall and femur, shin area).

When we were studied of indicators of oxidative modification of proteins, lipid peroxidation in blood plasma, antioxidant protection during this period, there were no significant changes in these parameters between the control and experimental groups.

Therefore, it can be concluded according to this observation and study, given the absence of significant changes in the relevant indicators (oxidative modification of proteins, lipid peroxidation in blood plasma, antioxidant protection), that the use of ozone therapy in patients with complicated forms of diabetes does not adversely affect this link pathological process.

The course of changes in the indexes of intoxication was also studied: leukocyte index of intoxication, hematological index of intoxication and sorption capacity of erythrocytes in patients of the main and control groups.

As a result of the study (at the time of admission, during treatment, at the time of discharge), it was found that the leukocyte index of intoxication in patients in the main and control groups was almost indistinguishable.

Regarding the indexes of hematological index of intoxication, which decreased during the treatment of patients of the main group, and different from the patients of the control group, in which this indicator increased sharply.

The result of the study of changes in the indicators of sorption capacity of erythrocytes showed that the level of sorption capacity of erythrocytes in patients in the main group during treatment, also decreased, compared with patients in the control group.

The increase in hematologic index of intoxication in patients in the control group can be regarded as a significant sign of existing intoxication in the body. The decrease in these indicators in the patients of the base group makes it possible to consider this as a low level of intussusception syndrome.

DISCUSSION

Therefore, given the dynamic course of indicators of oxidative modification of proteins, lipid peroxidation in blood plasma, antioxidant protection during this period, there were no significant changes in these parameters between the control and experimental groups. And leukocyte index of intoxication, hematological index of intoxication and sorption capacity of erythrocytes in patients of the main and control groups [8].

As a result of the study (at the time of admission, during treatment, at the time of discharge), it was found that the leukocyte index of intoxication in patients in the main and control groups was almost indistinguishable. Regarding the indexes of hematological index of intoxication, which decreased during the treatment of patients of the main group, and different from the patients of the control group, in which this indicator increased sharply [2].

The result of the study of changes in the indicators of sorption capacity of erythrocytes showed that the level of sorption capacity of erythrocytes in patients in the main group during treatment, also decreased, compared with patients in the control group [6].

The increase in hematologic index of intoxication in patients in the control group can be regarded as a significant sign of existing intoxication in the body. The decrease in these indicators in the patients of the base group makes it possible to consider this as a low level of intussusception syndrome [4].

Therefore, all the obtained results indicate that the use of ozone as a physical method in the complex treatment of patients with complications of diabetes mellitus does not

contribute to the negative impact of this method on the indexes of intoxication and homeostasis of blood. And it gives an opportunity to consider these results as a positive effect of ozone therapy in the complex treatment of patients with complicated forms of diabetes [5].

CONCLUSIONS

1. Therefore, the use of ozone in the treatment of patients with complicated forms of diabetes does not cause a negative impact on the dynamics of homeostasis and indicators of the level of intoxication of the body.
2. Ozone therapy has a beneficial effect on the course and wound process in patients with diabetes with purulent processes.

REFERENCES

1. Germanyuk T., Ivko I. Rational choice of monotherapy for the treatment of type 2 diabetes mellitus based on the pharmacoeconomical analysis. Science Rise. 2014; 4(5):69-72. doi: https://doi.org/10.15587/2313-8416.2014.32109 .
2. Atkinson M., Herrath M., Powers A. et al. Current concepts of the pathogenesis of type 1 diabetes — considerations for attempts to prevent and reverse the disease. Diabetes Care. 2015;38:979-988. doi: 10.2337/dc15-0144 .
3. Bolli G., De Vries J. New long-acting insulin analogs: from damp studies to clinical practice. Diabetes Care. 2015; 38: 541-543. dol: 10.2174/157339911794273892 .
4. Karatieieva S., Yurkiv O., Semenenko S. Evaluation of the use of ozone therapy in the treatment of inflammatory processes in diabetes mellitus in an experiment. Georgian medical news. 2016;10(259): 58-61.
5. Karatieieva S., Makarova O., Yurkiv O. Treatment of pyoinflammatory complications in diabetic patients with individual selection of ozone dose. Gorgian medical news. 2018; 2(275):91-94.
6. Kumar S., Chakravarty B. ABC/VED analysis of expendable medical stores at atertiary care hospital. Med J Armed Forces India. 2015; 71:24-27.
7. Andrews M., O'Malley P. Diabetes overtreatment in eldery individuals: risky business in need of better management .JAMA. 2014; 311: 2326-2327. doi: 10.1001/jama.2014.4563
8. Giwa A., Tayo F. Cost-effectiveness analysis of anti-diabetic therapy in a university teaching hospital. International Journal of Pharma Sciences and Research (IJPSR) .2014; 03: 82 – 91.
9. Mishchenko O., Iakovlieva L., Adonkina V. Pharmacoeconomic evaluation of fxed – dose triple combination for antihypertensive therapy in Ukraine. Journal of Health Policy Outcomes Research. 2014; 1: 66-74.
10. Mpondo B. Glycaemic control and glucose-lowering therapy in diabetic patients with kidney diseas. African Journal of Diabetes Medicine. 2014; 1: 12-16.
11. Sharma P., Sharma N., Parakh R. et al. Screening of prescriptions in patients of type –2 diabetes mellitus in a tertiary care teaching hospital. International journal of pharmaceutical research and bio-science. 2014; 3(1) : 401-409. doi: 10.20959/wjpps20173-8761

ORCID and contributionship:

Svitlana Y. Karatieieva: 0000-0003-1836-8337 [A,B]
Nataliia Y. Muzyka: 0000-0007-1976-8347 [C,F]
Karolina I. Yakovets: 0000-0002-1276-3337 [B]
Oksana V. Bakun: 0000-0008-1166-9544 [D]
Ksenia V. Slobodian: 0000-0005-1096-1092 [E]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Svitlana Yu. Karatieieva
Bukovinian State Medical University
2 Theatralna str. 58002 Chernivtsi, Ukraine
tel: +380662670935
e-mail: Karatsveta@gmail.com

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ORIGINAL ARTICLE

PECULIARITIES OF FREE RADICAL OXIDATION AND ANTIOXIDANT PROTECTION PARAMETERS OF THE ORAL FLUID IN CHILDREN WITH CHRONIC CATARRHAL GINGIVITIS WITH UNDERLYING DIABETES MELLITUS

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Liudmyla F. Kaskova[1], Valentyna A. Honcharenko[2], Oksana V. Klitynska[3]

[1]UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY, THE MINISTRY OF HEALTH OF UKRAINE, POLTAVA, UKRAINE

[2]BUKOVINIAN STATE MEDICAL UNIVERSITY, THE MINISTRY OF HEALTH OF UKRAINE, CHERNIVTSI, UKRAINE

[3]STATE HIGHER EDUCATIONAL ESTABLISHMENT OF UKRAINE «UZHHOROD NATIONAL UNIVERSITY», THE MINISTRY OF HEALTH OF UKRAINE, UZHHOROD, UKRAINE

ABSTRACT

The aim: Objective of the research was to study peculiarities of free radical oxidation and antioxidant protection parameters of the oral fluid in children with chronic catarrhal gingivitis with underlying diabetes mellitus.

Materials and methods: Oral fluid was examined in 105 children aged 12 including somatically healthy ones with intact periodontium and chronic catarrhal gingivitis, suffering from diabetes mellitus up to 5 years and more than 5 years with chronic catarrhal gingivitis. The parameters of lipid peroxide oxidation (level of diene conjugates, Malone dialdehyde) and antioxidant protection (activity of SOD and catalase, POM, whole protein; ceruloplasmin; HS-groups) of the oral fluid were determined.

Results: The results obtained are indicative of available probable difference in the examined parameters among children depending on their general state of health and periodontal tissue condition. The best parameters were found among somatically healthy children with intact periodontium. They deteriorated in children with chronic catarrhal gingivitis available irrespective of their general state of health. They reached the most critical level in children with diabetes mellitus lasting longer than 5 years.

Conclusions: The course of chronic catarrhal gingivitis in children, both somatically healthy and those suffering from diabetes mellitus, is associated with increased parameters of lipid peroxide oxidation and decreased enzymatic activity of the antioxidant protection system of the oral fluid. The most considerable changes are found in patients suffering from diabetes mellitus lasting more than 5 years.

KEY WORDS: lipid peroxide oxidation, antioxidant protection, oral fluid

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INTRODUCTION

Current views concerning genesis of dental diseases are based on a considerable role of membrane-stabilizing processes and mechanisms realizing them. There is a great amount of scientific studies that present arguments concerning lipid oxidation constantly available in all the organs and tissues under physiological conditions in small amounts . Under certain conditions, due to high chemical activity, radicals react with the major biological compounds disturbing their structure and functions. The antioxidant body system controls and inhibits all the stages of free radical reactions beginning from their initiation till the formation of hydroperoxides and Malone dialdehyde . Failure of the antioxidant protective mechanisms promotes an increased level of lipid peroxide oxidation (LPO) products in the body, resulting in realization of a non-specific complex of damage of the cellular membranes. Proteins play an important role in metabolic processes. When the processes of lipid peroxide oxidation are intensified, protein modification processes occur resulting in their fragmentation, denaturation, loss of their biological activity leading to disorders of regenerative processes in the tissues [1,2,3]. The role of lipid peroxide oxidation processes and the antioxidant system state in the development of different diseases including diabetes mellitus is confirmed [4,5]. At the same time, intensification of protein oxidative modification (POM) and LPO, decrease of the antioxidant protection system activity of the oral fluid are found in case of inflammatory diseases of the periodontal tissue [6, 7, 8].

THE AIM

Study peculiarities of free radical oxidation and antioxidant protection parameters of the oral fluid in children with chronic catarrhal gingivitis with underlying diabetes mellitus.

MATERIALS AND METHODS

To solve the task stomatological and laboratory examination of 105 children aged 12 was carried out. 65 children suffering from diabetes mellitus treated at the Children

Table I. Parameters of lipid peroxide oxidation of the oral fluid of children in the groups of observation (M±m)

Groups, number of children	Parameters of lipid peroxide oxidation (M±m)		
	POM (nmol/mg of protein)	Diene conjugate (mcM/ml of saliva)	Malone dialdehyde (mcmol/L)
Group 1 (n=22)	138,25±5,91	5,18±1,45	55,04±1,26
Group 2 (n=18)	176,61±4,01	8,47±0,93	70,59±1,36
p^1	<0,05	<0,05	<0,05
Group 3 (n=35)	202,57±2,35	15,53±0,73	91,68±1,19
p^2	<0,05	<0,05	<0,05
p^3	<0,05	<0,05	<0,05
Group 4 (n=30)	215,22±1,35	19,31±0,81	103,59±1,10
p^4	<0,05	<0,05	<0,05
p^5	<0,05	<0,05	<0,05
p^6	>0,05	>0,05	>0,05

Notes:
p^1 – difference probability of parameters in children of groups 1 and 2;
p^2 – difference probability of parameters in children of groups 1 and 3;
p^3 – difference probability of parameters in children of groups 2 and 3;
p^4 – difference probability of parameters in children of groups 1 and 4;
p^5 – difference probability of parameters in children of groups 2 and 4;
p^6 – difference probability of parameters in children of groups 3 and 4.

Table II. Parameters of the antioxidant protection of the oral fluid among children from the groups of observation (M±m)

Groups of children, number	Parameters of the antioxidant protection (M±m)				
	Whole protein (g/L)	SOD (UN/min* mg of protein)	HS-groups (pM/ mg of protein)	Ceruloplasmin (mg/L)	Catalase (nmol/ min* mg of protein)
Group 1 (22)	3,26±0,43	10,53±0,52	120,29±4,03	132,36±3,94	6,69±1,15
Group 2 (18)	8,23±0,91	7,36±0,11	85,98±1,69	106,99±4,19	4,71±0,56
p^1	<0,05	<0,05	<0,05	<0,05	<0,05
Group 3 (35)	14,58±0,13	5,03±0,13	67,59±0,76	91,3±3,77	2,31±0,03
p^2	<0,05	<0,05	<0,05	<0,05	<0,05
p^3	<0,05	<0,05	<0,05	<0,05	<0,05
Group 4 (30)	17,26±0,09	4,42±0,05	56,68±0,82	79,85±3,03	1,75±0,02
p^4	<0,05	<0,05	<0,05	<0,05	<0,05
p^5	<0,05	<0,05	<0,05	<0,05	<0,05
p^6	>0,05	>0,05	>0,05	>0,05	>0,05

Notes:
p^1 – difference probability of parameters in children of groups 1 and 2;
p^2 – difference probability of parameters in children of groups 1 and 3;
p^3 – difference probability of parameters in children of groups 2 and 3;
p^4 – difference probability of parameters in children of groups 1 and 4;
p^5 – difference probability of parameters in children of groups 2 and 4;
p^6 – difference probability of parameters in children of groups 3 and 4.

Endocrinological Department of the Municipal Institution "Regional Children Clinical Hospital" were examined including 35 individuals with diabetes lasting up to 5 years (group 3), and 30 ones with diabetes lasting longer than 5 years (group 4). The groups of comparison consisted of children without underlying pathology with healthy periodontium (22 individuals – group1) and with chronic catarrhal gingivitis (18 individuals – group 2).

Oral fluid of children from the groups of observation was the material for additional examination. Oral fluid of children for paraclinical examination was collected in the morning after rinsing the oral cavity twice with distilled water. The material was obtained by means of spitting out without stimulation of salivation in the volume of 5-6 ml. The material was transported and stored at -5°C. Before biochemical analyses the oral fluid was centrifuged during

15 minutes at a speed of 3000 rpm. Supernatant was used for examination. The following parameters were determined: the level of diene conjugates, Malone dialdehyde by N.D. Stalna's method [1977]; catalase activity with the use of ammonia molybdate [M.A. Koroliuk,1988]; SOD activity by S.Chevari's method [1985]; POM degree was assessed by the principle of interaction of amino acid protein residues with 2,4-dinitrophenylhydrazine with the formation of derivatives [E.E.Dubynina, S.O. Burmistrov,1995] in modification [I.F. Meshchyshen,1998]; whole protein; ceruloplasmin; HS-group.

The results obtained were statistically processed by Student method. The results with $p \leq 0,05$ were considered reliable.

RESULTS AND DISCUSSION

The parameters of lipid peroxide oxidation of the oral fluid of children from the groups examined possessed a reliable difference depending on the general health state and periodontal tissue condition (Table I). The best parameters were observed among somatically healthy children and intact periodontium. The worst parameters were found in children with chronic catarrhal gingivitis and diabetes mellitus lasting longer than 5 years.

Somatically healthy children with underlying diabetes mellitus and chronic catarrhal gingivitis present activation of the protein oxidative modification processes of the oral fluid in comparison with children having healthy periodontal tissue. POM degree of the oral fluid depends on the availability and duration of the underlying disease (diabetes mellitus) and periodontal tissue diseases available. The highest parameter was found among children suffering from diabetes mellitus longer than 5 years and have chronic catarrhal gingivitis. Protein oxidative modification degree in children from group 1 is by 1,28 times lower than in children from group 2. Thus, the course of chronic catarrhal gingivitis in somatically healthy children occurs together with deterioration of the parameter ($p<0,05$). The parameter increases in children suffering from diabetes mellitus lasting up to 5 years (by 1,15 times) and those with the duration of more than 5 years (by 1,22 times). A probable difference among the examined parameters depending on the duration of the underlying disease was not found, though numerical values differed.

The concentration of diene conjugates was the highest in patients with chronic catarrhal gingivitis and duration of diabetes mellitus of more than 5 years. In comparison with somatically and stomatologically healthy children this parameter 3,73 times increased (5,18±1,45 mcM/ml – in group 1 against 19,31±0,81 mcM/ml – in group 4). A similar tendency was found concerning Malone dialdehyde as well. The numerical values deteriorate in children with chronic catarrhal gingivitis, and it becomes of a maximum value in patients with inflammatory processes in the periodontal tissue and diabetes mellitus lasting for more than 5 years.

The antioxidant protection system functioning regulates free radical processes in the cells and tissues promoting ensuring free radical homeostasis in the whole body and oral cavity in particular. Examination of the parameters of antioxidant protection of the oral fluid in children from the groups of observation found their difference (Table II). The whole protein parameter is of a special attention, since it 5,3 times increases among the patients from group 4 in comparison with group 1, and the parameter of catalase activity 3,8 times decreases (6,69±1,15 nmol/min*mg of protein – in group 1 against 1,75±0,02 in group 4). Hydrogen peroxide is utilized by the intracellular iron-containing enzyme catalase which is functioning in a close relation with SOD. Therefore, examination of this parameter is important. Our studies found its decrease among the patients from groups 2,3 and 4 in comparison with healthy children (group 1). The worst parameter was found in children from group 4. It was 2,4 times lower than that of children from group 1. A probable difference of superoxide dismutase enzyme activity in children with different duration of diabetes mellitus was not found, but the parameters were worse in patients suffering from diabetes mellitus more than 5 years (5,03±0,13 UN/min* mg of protein in group 3 against 4,42±0,05 – in group 4).

The parameters of activity of HS-groups and ceruloplasmin decrease in case of inflammatory processes in the periodontal tissue, especially among children suffering from diabetes mellitus longer than 5 years.

A probable difference of POM, diene conjugates, Malone dialdehyde, whole protein, HS-group, ceruloplasmin, SOD activity, catalase of the oral fluid of children was found among different groups of observation. The highest rates were observed in children with a history of diabetes and suffering from it for more than 5 years. Increase of lipid peroxide oxidation parameters and decrease of enzymatic activity of the antioxidant protective system of the oral fluid were found in children with chronic catarrhal gingivitis available. The most considerable changes are found in patients with diabetes mellitus lasting for more than 5 years. Thus, the processes of antioxidant protection in children are influenced by the presence of somatic pathology and its duration, which is confirmed by the literature [2,6].

CONCLUSIONS

The results obtained force to regulate the processes of antioxidant protection in children with chronic catarrhal gingivitis especially with diabetes mellitus by means of development of therapeutic complexes which is a subject of further studies.

REFERENCES

1. Hnatiuk V.V. Gender and Age Peculiarities of Free Radical Oxidation and Antioxidant Protection with desynchronosis. Bulletin of Vinnytsia National Medical University. 2014; 2: 363-6.
2. Hovorukha O.Yu. The Value of Interaction of Lipid Peroxide Oxidation and Antioxidant Systems in the Development of Pathological Processes. Experimental and Clinical Medicine. 2016; 4 (73):10-14.
3. Tiazhka O.V. The State of Lipid Peroxide Oxidation and Antioxidant System in Children of Different Age. Perinatology and Pediatrics. 2016; 2(66): 101-5.

4. Mokryi V.Ya. Disorders of the Lipid Peroxide Oxidation System with Type 2 Diabetes Mellitus (literature review). International Journal of Endocrinology. 2015; 7(71):41-4.
5. Maydannyk V.H. The State of Lipid Peroxide Oxidation and Antioxidant System in Children Suffering from type 1 Diabetes Mellitus Depending on Chronic Complications Available .Issues of Clinical Pediatrics. 2018; 4(42): 26-8.
6. Palasiuk B.O. Lipid Peroxide Oxidation and Protein Oxidative Modification in the Oral Fluid of Preteen Schoolchildren with Chronic Catarrhal Gingivitis. Topical Issues of Modern Medicine. 2012; 4 (40):50-4.
7. Yanko N.V. The Mechanisms of Disorders of Free Radical Processes in the Periodontal Tissue of Rats under Conditions of Experimental Periodontitis and Development of Methods to Correct Them. Wiadomosci Lekarskie. 2016;3: 521-3.
8. Kaskova L.F. The Parameters of Calcium, Inorganic Phosphorus and Antioxidant Status of the Oral Fluid in Children with Chronic Catarrhal Gingivitis during the Period of Transitional Dentition. World of Medicine and Biology. 2013;1(36):187-8.
9. Ullah A., Khan A., Khan I. Diabetes mellitus and oxidative stress – a concise review. Saudi Pharm J. 2016;24(5):547-53.
10. Hodovanets O.I., Kotelban A.V. Osoblyvosti perebihu khronichnoho kataralnoho hinhivitu v ditei za umov tsukrovoho diabetu. Visnyk stomatolohii. 2016; 4: 60-5. (In Ukrainian).

«Improvement of methods of prevention and treatment of diseases of hard tissues of teeth and periodontal tissues on the background of somatic pathology in children, taking into account socio-economic factors and psycho-emotional state» (0119U102852).

ORCID and contributionship:
Lyudmyla F. Kaskova: 0000-0003-0855-2865 [A,F]
Valentyna A. Honcharenko: 0000-0001-7276-9052 [B,D]
Oksana V. Klitynska: 0000-0001-9969-2833 [C,E]

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CORRESPONDING AUTHOR
Valentyna A. Honcharenko
Bukovinian state medical university
2 Teatralna square, 58000 Chernivtsi, Ukraine
tel: +380677176001
e-mail: V.honcharenko_84@meta.ua

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ORIGINAL ARTICLE

NEGATIVE FACTORS OF MANAGEMENT ACTIVITIES OF THE SECURITY AND DEFENCE SECTOR REPRESENTATIVES AND DIRECTIONS OF THEIR OVERCOMING

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Ivan M. Okhrimenko[1], Nina V. Lyhun[1], Viktoriia P. Pryimak[2], Yaroslav I. Korol[3], Mykhailo S. Myroshnychenko[4]

[1]NATIONAL ACADEMY OF INTERNAL AFFAIRS, KYIV, UKRAINE

[2]KYIV FACULTY OF THE NATIONAL ACADEMY OF THE NATIONAL GUARD OF UKRAINE, KYIV, UKRAINE

[3]ODESA MILITARY ACADEMY, ODESA, UKRAINE

[4]KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

The aim is to substantiate the expediency of performing physical exercises by representatives of the security and defence sector to reduce the negative impact of management activities factors on their state of health.

Materials and methods: The peculiarities of professional activities of 82 officers of the National Police and the Armed Forces of Ukraine, who had military and special ranks of "lieutenant colonel", "colonel" and held senior positions (heads of faculties, departments, divisions, services and other units) were studied. Research methods: theoretical analysis and generalization of literature sources, pedagogical observation, interviews, questionnaires.

Results: It was found that management activities are characterized by a number of negative factors, which negatively affect the body of management level officers. With systematic interference, they significantly worsen the state of officers' health and negatively affect the effectiveness of their management activities.

Conclusions: Physical education has significant potential in improving the efficiency of professional activities of management level officers, strengthening their health and enhancing working ability. The direction of improving physical training of management level personnel is determined as increasing the amount of general physical training, conducting classes with different orientations taking into account the age group, service load and training within the working time pattern.

KEY WORDS: negative factors, management activities, physical education, health

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INTRODUCTION

In recent years, there has been a tendency towards reduction of the level of physical fitness and health of the population of Ukraine. Representatives of the security and defence sector i. e. servicemen and police officers are not an exception. This is especially true for management level, whose professional activities are characterized by a significant reduction in physical exercises, high intellectual loads, stresses, responsibility for decision-making and more.

The works [1-5] point out a deterioration of the state of health and the level of physical fitness of the male population of Ukraine in recent years under conditions of social, economic and political instability: the rate of depopulation in Ukraine ranks the 2nd place in the world, the 1-2nd place in Europe for mortality from cardiovascular diseases; the average life expectancy of men in Ukraine is 10-12 years lower than in the European Union countries; more than 70 % of the adult male population have low and below average levels of physical health. The probability of death of working age men is 38.4 %, which is one of the worst indicators among the civilized countries of the world. More than 80 % of fatalities in middle-aged and elderly people at the present time account for hypertension, atherosclerosis, and obesity.

Modern management activities of the security and defence sector representatives take place under specific conditions associated with significant psychological load, constant growth of information, high level of responsibility for decisions, overload of the intellectual sphere, high nervous and emotional stress, long-term limitation of motor performance and preservation of static postures. These factors lead to a decrease in mental and physical performance, deterioration of the body's major systems (especially cardio-respiratory), the appearance of excess weight, and with systematic action can worsen health and cause various diseases (hypertension, coronary heart disease, etc.) [6-8].

According to many scientists [9-11] physical education is one of the ways to overcome the negative impact of adverse factors of professional, including management activities. Physical education is designed to increase the level of physical fitness, improve physical development and functional capabilities of servicemen and police officers, strengthen their health, improve psycho-emo-

tional state, enhance working ability, expand professional performance, provide a range of recreational and rehabilitation activities.

THE AIM

The aim of this study is to substantiate the expediency of performing physical exercises by representatives of the security and defence sector to reduce the negative impact of management activities factors on their state of health.

MATERIALS AND METHODS

The research was conducted at the National Academy of Internal Affairs (Kyiv, Ukraine) and the Military Academy (Odessa, Ukraine) in 2016-2020. The peculiarities of professional activities of 82 officers of the National Police and the Armed Forces of Ukraine, who had military and special ranks of "lieutenant colonel", "colonel" and held senior positions (heads of faculties, departments, divisions, services and other units) were studied.

Research methods: theoretical methods included the analysis and generalization of literature sources, comparison was used to investigate the theoretical foundations of the problem, pedagogical experience of physical education implementation in the process of professional activities of specialists to improve their health, working ability, longevity, increase the body's resistance to negative factors of professional activities; empirical methods included pedagogical observation, interviews, questionnaires in order to clarify the characteristics of the professional activities of management level officers, finding ways to apply physical exercises by representatives of the security and defence sector to reduce the negative impact of management activities factors on their state of health.

The research was performed according to the requirements of the Regulations on Academic Honesty at the National Academy of Internal Affairs, which were developed on the basis of Ukrainian and world experience of ethical rulemaking. The consent to participate in the research was obtained from all subjects.

RESULTS

One of the main negative factors of modern management activities is low motor performance. Low motor performance results in various disorders of the body's systems, metabolism, and the appearance of excess weight, which adversely affects the state of health and causes various diseases [12]. First of all, the cardiovascular system suffers (atherosclerosis, hypertension, coronary heart disease, etc.), the condition of the musculoskeletal system deteriorates. As a result, there is a phenomenon of detraining (deterioration of the bear of functional loads). Low motor performance, nervous and emotional stress lead to chronic stress [13]. Low motor performance adversely affects the condition of the muscular system. Muscles become flabby and shrink in size. The pectoral muscle sling does not perform its main function (the spine is not held in a normal position), which leads to a deterioration in the brain blood supply and, consequently, a decrease in mental and physical working ability of management level officers. Atrophic changes in part of the muscle fibres are also noticeable, and the layer of adipose tissue between them increases. Stability and reliability of coordination of movements are lost, force and endurance of muscles decrease. Muscle atrophy negatively affects the adaptive mechanisms of blood circulation during physical load and ultimately negatively affects the officers' health and their ability to work [14].

In addition management level officers experience high nervous and emotional stress. The scientists [13, 15] claim that there is a decrease in working ability, increased heart rate, respiration and pressure, difficulty distributing attention, narrowing the scope of attention and memory, increasing the number of erroneous actions, and rapid fatigue as a result of nervous and emotional stress. A decrease in the functional capabilities of the body and its reserves lie at the heart of fatigue. One of the first signs of fatigue is a decrease in mental activity since the service activities of management level officers are closer in nature to the types of mental and intellectual work. Memory, concentration, distribution and switching of attention deteriorate in case of fatigue. The initial period of fatigue is characterised by a noticeable decrease in muscle strength and endurance, impaired coordination of movements, as well as the speed of reactions. Fatigue can also be manifested in the appearance of irritability, deterioration of general physical and mental state.

It would seem that the need for constant physical improvement of management level officers disappears in connection with the reduction of muscular energy expenditure and the increase of mental load. However, the studies show that reducing the share of muscle energy expenditure in the process of work in many cases does not reduce, but increases the requirements for physical fitness of specialists [16]. Thus, adverse factors dramatically impair the physical condition, working ability and health of management level officers and the effectiveness of their overall service activities. Therefore, the importance and role of physical education in improving the effectiveness of management activities, strengthening the health and tolerance of officers towards various negative factors is increasing as never before. Physical education, solving its basic task i. e. the development of basic physical qualities, can solve aspect problems such as the formation of emotional stability, health promotion, formation of tolerance towards professional activities negative factors, etc. [2, 9, 19, 17].

We conducted a survey of 82 management level officers in order to study the observance of a healthy lifestyle, performing exercises to overcome the negative factors of management activities by the management level officers. The results of the analysis of the answers regarding a healthy lifestyle keeping by management level officers showed that only 10.3 % of officers regularly do morning exercises every day (23 % – sometimes and 66.7 % – never); 85.7 % of

officers get to the place of their service by public transport or by their own car and only 14.3 % take a walk (mostly officers who live in dormitories near the place of their service). On weekends and holidays, preference is given to passive types of physical activities and recreation (watching TV, working on a computer, improving the professional level, etc.) by 41.3 % officers; 37.3 % of officers spend their free time with family and friends and only 9.5 % of officers engage in exercises and sports, practice hardening techniques, and prefer hiking. There is much tension around the issue of obsessive habits, including smoking. Thus, the question concerning tobacco abuse revealed the fact that more than 50 % of management level officers smoke, whereupon 20.6 % of surveyed officers use no more than half a pack of cigarettes a day, 30.9 % – one pack or more a day; only 6.7 % of management level officers answered that they do not smoke. The analysis of the answers to the question concerning the application of health promotion factors showed that many management level officers (67.1 %) rarely or almost never use factors that contribute to the preservation and promotion of health.

Irritability, depression, tiredness, headache and other symptoms due to the negative effect of management activities factors at the end of the working day (week) are experienced by 88.9% of officers (22.2 % – sometimes, 51.6 % – often; 15.1 % – always) and only 11.1 % officers never experience such symptoms. It is important to note that the majority of officers, who always have similar symptoms and complaints, belong to the older age groups (over 45 years old, 94.7 %), which indicates that the negative impact of management activities increases with age along with the risk of various diseases occurrence.

The effectiveness of physical training in the formation of the necessary qualities of specialists depends on the variety of means used, their scope and direction. The studies [5, 7] showed the need for integrated use of various means of physical education to optimize the physical well-being of servicemen, whose activities take place in conditions of limited motor performance. The peculiarity of the organization of physical education of servicemen, whose activities have a pronounced hypodynamic nature, is the use of general physical training means with the predominant use of strength and endurance exercises. The scientists [10, 11] found that the overall level of development of officers' physical qualities depends largely on the development of endurance and strength. Therefore, physical education classes for management level officers, whose activities are accompanied by the influence of insufficient motor performance and other negative factors, should be built on the principle of versatile training.

The use of physical exercises to restore working ability during mental work is of particular interest. A high level of mental working ability is maintained provided that there is constant optimal excitability of the respective areas of the cerebral cortex. As a result of long-term mental work there are inhibitory processes in the higher parts of the brain that cause a decrease in attention, memory, ability to creatively analyse information, and cause making errors. Periodic transition to physical exercises in the form of classes can speed up recovery of working ability, relieve fatigue feeling, have a general health effect.

Physical exercises help to improve the mobility of nervous processes in the course of mental work and thus create the conditions for longer-term working abilities. The author also notes that the stability of mental work depends on the level of general physical fitness. Working ability indicators were less stable in people having a low level of general physical fitness. In the case of performing exercises in the process of mental activities, their selection and duration of working performance depend on the nature of mental work. If it consists of the usual mental operations that do not contain complex analytical components, then in this case, moderate loads of increased duration will not cause a decrease in the productivity of the main work. But if the mental work is creative, associated with the analysis of a wide range of information, then in this case, it is not allowed disrupting the system of intercentral relations by switching to intense muscle activities. Here the positive effect is achieved by mild exercises, the performance of which does not violate the dynamic stereotype of the main work in the cortex. Excessive emotional impact, which can also increase the excitability of the central nervous system and complicate further inclusion in mental work, should also be avoided when performing exercises in the process of mental activities.

The content of the complexes that are performed in the process of mental work should include exercises that increase resistance to long-term maintenance of a monotonous posture and physical inactivity (general development exercises and exercises with your own body weight); exercises that slightly excite the central nervous system and have a preventive effect (hiking, slow running, etc.); exercises that develop the muscles of the back and abdomen, as well as stimulate blood circulation; special exercises that focus the nervous system on future work.

DISCUSSION

Management activities require officers to maintain a long-lasting permanent working position. However, its long-term maintenance (static mode) is often more tiresome than work, accompanied by little motor performance. A disturbance of correlation between pulmonary ventilation and blood supply of lungs owing to unevenness of breath and decrease in saturation of blood with oxygen occurs in the conditions of static work of average complexity. As a result, the phenomenon of oxygen starvation (hypoxia) and the accumulation of carbon dioxide are developed in the body [8, 11, 18]. Scientists found [12, 13, 19, 20] that only some servicemen endure 4-hour immobility in a "sitting" posture without complaints of exhaustion, in which fatigue is localized in the muscles of the neck, back and legs; the cardiovascular system suffers the most. The expenditure of neuromuscular energy to maintain posture is greater the more uncomfortable posture is. Blood circulation deteriorates, stagnation occurs, and working ability is reduced in

the case of long-term maintenance of a monotonous posture.

Management level officers spend most of their office hours indoors in a forced posture (usually sitting), which convincingly demonstrates the effect of a significant number of negative factors on the officers' body systems, including: hypodynamics and hypokinesia, working hours and weeks, nervous and emotional stress, mental overload and stress and others. In addition, obsessive habits (mostly smoking) and lack of time for physical training do not provide the necessary level of physical fitness and health of management level officers for the effective implementation of their management activities. Such activities occur over many years, and with age, these factors lead to a significant decrease in mental and physical performance, deteriorating the state of health and the functioning of major body systems.

We set that the tasks of physical education of management level personnel of the security and defence sector of Ukraine are to increase the level of general physical fitness, strengthen health, improve the functionality of the body's major systems, prevent diseases, prolong professional longevity, ensure a high level of intellectual work capacity, enhance resistance to negative factors of management activities.

It is established that the use of physical exercises in the daily routine of management level officers should be aimed at solving the following tasks:

- in the process of management activities – to change the dynamics of subcortical processes, to create an optimal background of excitability; to improve cerebral blood circulation and metabolism, actively influencing the cardiovascular system; to carry out preventive influence on the musculoskeletal system and digestive organs; to eliminate stagnation (especially in the pelvis and lower extremities); to ensure high mental working ability (to improve memory, thinking, attention); to improve emotional state;
- at the end of the working day – to develop and improve physical qualities (formation of general physical fitness); to strengthen muscles (to form a pectoral muscle sling); to strengthen health, improve the activities of the cardiovascular, respiratory and other systems of the body; to reduce the impact of physical inactivity and other adverse factors of service activities; to reduce fatigue due to the inclusion in the vigorous activities of muscle groups that did not participate in the work process, and to recover working ability.

CONCLUSIONS

It was found that management activities are characterized by a number of negative factors (low motor performance, stress, psychologic stress, monogony, etc.), which negatively affect the management level officers' bodies. With systematic interference, they significantly worsen the state of officers' health and negatively affect the effectiveness of their professional (management) activities. Physical education has significant potential in improving the efficiency of professional activities of management level officers, strengthening their health, enhancing working ability and prolonging professional longevity.

It was established that the means of physical training of management level officers of the security and defence sector of Ukraine should be simple, available and at the same time effective and health-improving exercises of general physical training.

The direction of improving physical training of management level personnel of the security and defence sector of Ukraine is determined as increasing the amount of general physical training, conducting classes with different orientations, taking into account the training within the working time pattern.

REFERENCES

1. Apanasenko G. L. Knygha o zdorovj'e [Health Book]. Kyev: Medknygha; 2007, 132 p. (In Russian).
2. Prontenko K., Bloshchynskyi I., Griban, G. et al. Formation of readiness of future physical culture teachers for professional activity. Universal Journal of Educational Research. 2019; 7(9): 1860-1868. doi: 10.13189/ujer.2019.070903.
3. Gruzieva T. S., Galiienko L.I., Pelo I. M., et al. Health and lifestyle of students' youth: status, problems and ways of solution. Wiad Lek. 2018; 71 (9): 1753-1758
4. Borys I. Palamar, Halyna O. Vaskivska, Svitlana P. Palamar. Didactic aspects of cognition of human as a bio-psycho-socio-cultural personality. Wiad Lek. 2017; 70 (5): 959-963
5. Griban G., Kobernyk O., Terentieva N. et al. Formation of health and fitness competencies of students in the process of physical education. Sport Mont. 2020; 18(3): 73-78. doi: 10.26773/smj.201008.
6. Prontenko K., Griban G., Prontenko V. et al. Kettlebell lifting as a means of physical training cadets in higher military educational institution. J Phys Educ Sport. 2017; 17 (4): 2685-2689. doi:10.7752/jpes.2017.04310.
7. Burley S.D., Drain J.R., Sampson J.A., Groeller H. Positive, limited and negative responders: the variability in physical fitness adaptation to basic military training. J Sci Med Sport. 2018; 21 (11): 1168-1172. doi:10.1016/j.jsams.2018.06.018.
8. Aandstad A., Hageberg R., Saether O., Nilsen R. Change in anthropometrics and aerobic fitness in Air Force cadets during 3 years of academy studies. Aviat Space Environ Med. 2012; 83 (1): 35-41. doi: 10.3357/ASEM.3069.2012.
9. Mozolev O., Halus O., Bloshchynskyi I. et al. Human resources management of educational development in sphere of physical culture and sports in Ukraine: comparative analysis (1992–2016). J Phys Educ Sport. 2019; 19 Suppl 1: 185-192. doi:10.7752/jpes.2019.s1028.
10. Prontenko K., Griban G., Prontenko V. et al. Health improvement of cadets from higher military educational institutions during kettlebell lifting activities. J Phys Educ Sport. 2018; 18(1): 298-303. doi: 10.7752/jpes.2018.01040.
11. Santtila M., Häkkinen K., Karavirta L., Kyrolainen H. Changes in cardiovascular performance during an 8-week military basic training period combined with added endurance or strength training. Mil Med. 2008; 173 (12): 1173-1179. doi: 10.7205/MILMED-D-16-00266
12. Melnykov A., Iedynak G., Galamandjuk L. et al. Factors that influence change in cadets' physical preparation during the first half of study at the military academy. J Phys Educ Sport. 2018; 18 (2): 781-786. doi:10.7752/jpes.2018.02115.
13. Henning P.C., Park B-S., Kim J.S. Physiological decrements during sustained military operational stress. Mil Med. 2011; 176 (9): 991-997. doi: 10.7205/milmed-d-11-00053.

14. Prontenko K., Griban G., Aloshyna A. et al. The physical development and functional state as the important components of the students' health. Wiad. Lek. 2019; 72(12a): 2348-2353. doi: 10.36740/WLek201912115.
15. Griban G., Yavorska T., Tkachenko P. et al. Motor activity as the basis of a healthy lifestyle of student youth. Wiad. Lek. 2020; 73(6), 1199-1206. doi: 10.36740/WLek202006123.
16. Prontenko K., Griban G., Bloshchynskyi I. et al. Improvement of students' morpho-functional development and health in the process of sport-oriented physical education. Wiad Lek. 2020; 73(1): 161-168. doi: 10.36740/WLek202001131.
17. Griban G., Prontenko K., Zhamardiy V. et al. Professional stages of a physical education teacher as determined using fitness technologies. J Phys Educ Sport. 2018; 18(2): 565-569. doi:10.7752/jpes.2018.02082.
18. Prontenko K., Griban G., Dovgan N. et al. Students' health and its interrelation with physical fitness level. Sport Mont. 2019; 17(3): 41-46. doi 10.26773/smj.191018.
19. Mozolev O., Bloshchynskyi I., Prontenko K. et al. Influence of fitness techniques integration on the development of physical qualities and morpho-functional state of adult females. Human Movement. 2021; 22(1): 57-65. https://doi.org/10.5114/hm.2021.98465.
20. Prontenko K., Griban G., Aloshyna A. et al. Analysis of cadets' endurance development at higher military educational institutions during the kettlebell lifting training. Sport Mont. 2019; 17(2): 3-8. doi 10.26773/smj.190601.

ORCID and contibutionship:
Ivan M. Okhrimenko: 0000-0002-8813-5107 [A,B,F]
Nina V. Lyhun: 0000-0002-3575-4350 [B,E]
Viktoriia P. Pryimak: 0000-0002-2962-1113 [C,E]
Yaroslav I. Korol: 0000-0003-2274-5858 [A,D]
Mykhailo S. Myroshnychenko: 0000-0002-6920-8374 [B,E]

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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

CHARACTERISTICS OF THE VAGINAL MICROBIOTA, CERVICAL AND UTERINE FLORA IN WOMEN WITH THE PAST HISTORY OF SEXUALLY TRANSMITTED INFECTIONS

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Olena I. Krotik
SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION OF THE MINISTRY OF HEALTH OF UKRAINE, KYIV, UKRAINE

ABSTRACT

The aim: To identify the characteristics of the vaginal microbiota, cervical and uterine flora in women with the past history of sexually transmitted infections.

Materials and methods: A prospective study and the analysis of the vaginal microbiota, cervical and uterine flora were conducted in 96 women of reproductive age with infertility after the past history of STIs, who planned to get pregnant using ART. Together they made up the group I (main group) and the CG included 30 women of reproductive age, who planned pregnancy without a compromised reproductive history.

Results: Nonspecific vulvovaginitis ($\chi 2 = 31.6$; $p < 0.001$) was diagnosed in 63 (63.6%) patients of the group I, vulvovaginal candidiasis – in 17 (17.7%). *Lactobacilli* in a vaginal discharge in representatives of the CG and group I – 96.7% and 29.1%, respectively ($\chi 2 = 20.3$; $p < 0.001$). Opportunistic pathogens, BV-associated microorganisms and obligate anaerobes in the next titers: 10x5 CFU/ml and higher, CG < 10x3 CFU/ml were found in 68 (70.8%) women of the group I. *N. gonorrhoeae* was detected in 2 (2.1%) patients of the group I. An intense leukemoid reaction – in 59 (61.5%) women of the group I. The rate of cervical contamination with these pathogens ranged from 10x3 to 10x4 CFU/ml in 35 (36.5%) women of the group I, in 61 (63.5%) – titers of opportunistic pathogens were > 10x4 CFU/ml. Pathogenic bacteria of the cervical canal, represented by obligate anaerobes, was detected in 70 (72.9%) cases, where 11 (11.4%) patients were diagnosed with *Chlamydia trachomatis*, 2 (2.1%) – *N.gonorrhoeae*. *Chlamydia trachomatis* and *Mycoplasma genitalium* were registered in 11 (11.5%) and 19 (19.8%) cases, respectively, and were not detected in controls. *Ureaplasma urealyticum* in the cervical canal outnumbered the diagnostic level in women: 17.7% – group I and 6.7% – CG, respectively ($\chi 2 = 7.9$; $p < 0.01$). *N.gonorrhoeae and Trich. vaginalis* were detected in 2.1% and 6.25% of women in the group I, respectively. Persistent urogenital infection – the combination of opportunistic and common pathogens – prevailed in 59 (60.2%). Associations of obligate anaerobes and *Chlamydia trachomatis* were found in 69 (71.8%) of them, microaerophiles – in 29 (30.2%), the combination of various species of opportunistic pathogens – in 14 (14.6%); the growth of monocultures of opportunistic pathogens, in titers coming to 10^6-10^8, was recognized in 19 (19.8%). The growth of monoculture, represented by group B *Streptococcus* was detected in 8 (8.3%) cases, *Enterococcus faecalis* – in 12 (12.5%), *Mycoplasma genitalium* – in 17 (17.7%). The analysis of PCR test results enabled to detect associations of different species of viruses with *Myco- and Ureaplasma*, *Chlamydia* in 23 (23.9%) patients of the group I. What should be noted is the predominance of *Herpes* simplex over CMV infection: 7 (7.3%) and 2 (2.1%), respectively ($p < 0.05$).

Conclusions: The predominance of the mixed microbiota, represented by various associations of opportunistic and common pathogens, was identified in infertile women with the past history of STIs as a result of microbial culturing of the samples from the uterine cavity. Chronic inflammation in endometrium is caused by a persistent viral-bacterial infection in the uterine cavity, which differs in the species composition from the microbiota of the lower genital tract.

KEY WORDS: sexually transmitted infections (STIs), pelvic inflammatory disease, infertility, microbiota, assisted reproductive technologies

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INTRODUCTION

Sexually transmitted infections (STIs) deeply affect sexual and reproductive health worldwide and are ranked among top 5 disease categories, in case of which adults usually seek medical care [1, 2]. More than 1 million people get a sexually transmitted infection daily. It is estimated that 500 million people annually get one of four STIs: chlamydia infection, gonorrhea, syphilis and trichomoniasis. More than 530 million people have HSV-2 [3].

In particular cases, STIs can have serious consequences for reproductive health in addition to the direct effects of the infection itself [3, 4-6]. STIs such as gonorrhea and chlamydia infection are the leading causes of pelvic inflammatory disease, unfavorable outcomes of pregnancy and infertility (WHO, 2018). And also tend to be the common cause of failures in case of using assisted reproductive technologies (ART) [7].

As for the complications in case of infecting with sexually transmitted pathogens, the first thing to be considered is impacted fertility represented by tubo-peritoneal, endocrine and uterine factor infertility. Tubo-peritoneal factor infertility appears due to structural or functional disorder of tubal patency as a result of inflammation. Endocrine factor infertility – due to ovulatory dysfunction and luteal insufficiency, occurring in case of oophoritis. Uterine factor infertility – due to abnormal endometrial maturation in case of endometritis. Ectopic pregnancy and complications in intrauterine pregnancy in the form of habitual miscarriage (13-25%), prelabor rupture of membranes, premature birth, postpartum endometritis, chorioamnionitis and placental inflammation, perinatal infection

Table I. Vaginal microbiota in patients examined (abs. n., %)

State of microbiota	CG n=30		Group I n=96		χ^2	P
	Abs. n.	%	Abs. n.	%		
Normal microbiota	25	83.3	6	6.2	80.1	<0.001
Intermediate type	2	6.7	5	5.2	29.4	<0.001
Bacterial vaginosis	2	6.7	5	5.2	0.78	>0.1
Nonspecific vulvovaginitis	1	3.4	63	63.6	31.6	<0.001
Vulvovaginal candidiasis	-	-	17	17.7	-	-

Table II. Microbiota of the vaginal discharge in patients examined (abs. n., %)

Microbiota	CG n=30		Group I n=96		$\chi 2^2$	P
	Abs. n.	%	Abs. n.	%		
Lactobacillus sp.	29	96.7	28	29.1	20.3	<0.001
Corynebacterium sp.	17	56.7	34	35.4	4.62	<0.05
Candida albicans	-	-	10	10.4	-	-
Bacteroides sp.	7	23.3	44	45.8	4.5	<0.05
Staphylococcus sp.	9	30.0	53	55.2	4.48	<0.05
Gardnerella vaginalis	10	33.3	56	58.3	4.35	<0.05
Mobyluncus	3	10.0	25	26.0	3.94	<0.05
N.gonorrhoea	-	-	2	2.1	-	-
Prevotella	10	33.3	43	44.8	5.5	<0.05
Peptostreptococcus sp.	6	20.0	45	46.9	5.9	<0.05
Streptococcus sp.B	10	33.3	54	56.25	3.97	<0.05
Enterococcus sp.	11	36.7	44	45.8	4.07	<0.05
Enterococcus faecalis	11	36.7	41	42.7	5.62	<0.05
E. coli	-	-	9	9.4	-	-
Atopobium vaginae	-	-	9	9.4	-	-

Table III. Microbiota of the cervical canal in women examined (abs.n., %)

Microscopic examination	CG n=30		Group I n=96		χ2	P
	Abs. n.	%	Abs. n.	%		
Leukocytes up to 10 in the field of view	27	90.0	37	38.5	26.4	<0.001
Leukocytes >25 in the field of view	3	10.0	59	61.5	6.75	<0.01

(8-12%) and stillbirth happen at times [8, 9]. Mother-to-child transmission of STIs can lead to stillbirth, neonatal death, low birth weight, prematurity, sepsis, pneumonia, neonatal conjunctivitis, and birth defects [9-11].

Thus, research on characteristics of the vaginal microbiota, cervical and uterine flora in women with the past history of sexually transmitted infections will enhance the effectiveness pre-pregnancy care before ART cycles in order to recover reproductive health in patients of this group.

THE AIM

To identify the characteristics of the vaginal microbiota, cervical and uterine flora in women with the past history of sexually transmitted infections, who are planning to implement their reproductive function by means of ART programs, were studied and analyzed.

MATERIALS AND METHODS

We examined 96 infertile women of reproductive age with the past history of STIs, who planned pregnancy assisted by ART, at the clinical site of Kyiv City Center for Reproductive and Perinatal Medicine over the period of 2018-2020 (clinical site of the department of Obstetrics, Gynecology and Reproductology of Shypuk NMAPE of the Ministry of Health of Ukraine). They comprised the group I (main group) and CG included 30 women of reproductive age,

who are planning pregnancy without a compromised reproductive history.

When studying the mocrobiota of the genital tract we stuck to the step-by-step approach: vagina, cervical canal, uterine cavity (endometrium). A comprehensive microbiological analysis, involving microscopy of gram- stained smears, culturing and molecular DNA analysis of sexually transmitted pathogens using PCR enabled to assess the spectrum of pathogens.

RESULTS

In order to study the etiology of inflammation in the uterine cavity, a comprehensive microbiological analysis of the vaginal and cervical discharge was conducted in all women examined. The vaginal microbiota was assessed according to a comprehensive microbiological analysis of the vaginal discharge. The microbiota in a gram-stained vaginal smear was assessed and cultured (Tables I and II).

In controls in 25 (83.3%) cases, microecological parameters of a vaginal smear complied with the standards of the reproductive age range: first-degree purity of the vaginal smear was identified when assessing the microbiota, leukocyte number was less than 10 in the field of view, the epithelium was represented by cells of superficial and intermediate layers. The microbiota was homogenous in composition (only *Lactobacillus* morphotypes prevailed) and medium in number (right up to 100 microbial cells in the field of view). Nonspecific vulvovaginitis ($\chi2 = 31.6$; $p < 0.001$) was diagnosed in 63 (63.6%) patients of the group I, the findings in smears were indicative of vulvovaginal candidiasis in 17 (17.7%). The rate of bacterial vaginosis in both groups of patients was not distinctive ($\chi2 = 0.78$; $p > 0.10$).

In patients from the CG when culturing a vaginal discharge, *Lactobacilli* were predominant in contrast to the findings in the group I: 96.7% and 29.1%, respectively ($\chi2 = 20.3$; $p < 0.001$). Among patients of the CG, *Lactobacilli* were isolated from the monoculture in 6 (20.0%), in 23 (76.7%) lactic acid bacteria were isolated as the association with opportunistic pathogens, the titers of which did not exceed 103-104 CFU/ml: enterococci were registered in 11 (36.7%) patients, corynebacteria in 17 (56.7%), staphylococci in 9 (30.0%), group B *Streptococcus* in 10 (33.3%).

In 68 (70.8%) infertile women with the past history of STIs, the specimen composition of the vaginal microbiota was characterized by a significant decrease in the frequency of lactobacillus isolation, in association with the positive growth of facultative anaerobes, which comprise opportunistic pathogens (*staphylococci, streptococci, BV-associated microorganisms (Gardnerella, Mobilincus, Atopobium vaginae)* and *obligate anaerobes (bacteroids, peptostreptococci, fusobacteria)* in elevated titers coming to 105 CFU/ml and higher, as compared to the values in the CG, where the titers of opportunistic pathogens did not exceed 103 CFU/ml. *N. gonorrhoeae* was detected in 2 (2.1%) patients of the group I, while neither case in women of the CG.

A comprehensive microbiological analysis of the cervical discharge in patients of both groups consisted of microscopic, microbial culturing and the assessment of the specimen composition of pathogens by means of the PCR test. Tables III and IV represent the results of microscopic examination of the cervical discharge, sum frequency of microorganism isolation, qualitative and quantitative composition of the microbiota of the cervical canal.

Thus, an intense leukemoid reaction was observed in 59 (61.5%) infertile women with the past history of STIs. It significantly differs from equivalent parameters in women from the CG, who, in 27 (90.0%) cases, had the number of leukocytes up to 10 in the field of view ($\chi2 = 26.4$; $p < 0.001$). Microbial culturing of the cervical discharge indicated that the growth of microbiota in all samples was not detected. The growth of opportunistic pathogens, originating from facultative anaerobes, was diagnosed in all cases of microbial culturing in patients of the group I. In 21 (21.4%) of these patients, the cervical microbiota was represented by monocultures of group B *Streptococcus*, in 19 (19.8%) – *Enterococcus faecalis*, in 21 (21.9%) – as a part of the associations of opportunistic pathogens. The quantitative assessment showed that the rate of cervical colonization with these pathogens ranged from 103 to 104 CFU/ml in 35 (36.5%) women of the group I, titers of opportunistic pathogens were increased (>104 CFU/ml) in 61 (63.5%). Pathogens, represented by obligate anaerobes, were found in 70 (72.9%) cases in the process of culturing the cervical discharge, and *Chlamydia trachomatis* was found in 11 (11.4%) of these cases in the group I, *N.gonorrhoeae* – in 2 (2.1%).

The findings of the PCR test, when studying the species composition of microorganisms in the cervical canal, indicate the high frequency of detecting pathogens associated with STIs in patients of the group I, comparing to the findings in patients of the CG. The cases when *Chlamydia trachomatis* and *Mycoplasma genitalium* were registered came to 11 (11.5%) and 19 (19.8%), respectively, while they were not detected in controls. The rate of cervical contamination with *Ureaplasma urealyticum*, based on the findings of the PCR test, outnumbered the diagnostic level in women: 17.7% – group I and 6.7% – CG, respectively ($\chi2 = 7.9$; $p < 0.01$). *N.gonorrhoeae* and *Trich. vaginalis* were detected in 2.1% and 6.25% of women of the group I, respectively. The frequency of human papillomavirus in women of both groups was not distinctive ($p > 0.05$) (Table V).

The microbiome of an endometrial biopsy in the main group was assessed by means of culturing and the PCR test. When assessing the findings of a microbiological analysis in infertile women with the past history of STIs, endometrial urogenital infection was found in endometrial samples in all cases, however the species composition of microorganisms differed from those detected in the vaginal and cervical discharge.

When culturing the content of the uterine cavity (Table VI), persistent urogenital infection was found to prevail in 59 (60.2%) cases. It was represented by various combinations of opportunistic and common pathogens. Asso-

Table IV. Microbiota of the cervical canal in patients of the group I (abs. n., %)

Types of microorganisms	Group I n=96	
	Abs. n.	%
Group B Streptococcus	19	19.8
Enterococcus faecalis	21	21.9
Group B Streptococcus + Enterococcus faecalis	23	23.9
Prevotella melaninogenica	28	29.1
Peptostreptococcus sp.+Prevotella melaninogenica	31	32.3
Chlamydia trachomatis	11	11.4
Ureaplasma urealyticum	49	51.0
Mycoplasma genitalium	18	18.7
N.gonorrhoeae	2	2.1

Table V. PCR test of the cervical discharge in women examined (abs. n., %)

Microbiota	CG n=30		Group I n=96		χ2	P
	Abs. n.	%	Abs. n.	%		
Trichomonas vaginalis	-	-	6	6.25	-	-
Chlamydia trachomatis	-	-	11	11.5	-	-
Ureaplasma urealyticum	2	6.7	17	17.7	7.9	<0.01
Mycoplasma genitalium	1	3.3	19	19.8	7.9	<0.01
N.gonorrhoeae	-	-	2	2.1	-	-
Herpes simplex virus I, II	-	-	4	4.2	-	-
Cytomegalovirus	-	-	3	3.1	-	-
HRV	2	6.7	18	18.7	3.4	>0.05

Table VI. Flora of the uterine cavity in patients of the group I (abs. n., %)

Microorganisms	Group I n=96	
	Abs. n.	%
Group B Streptococcus	8	8.3
Enterococcus faecalis	12	12.5
Group B Streptococcus + Enterococcus faecalis	14	14.6
Prevotella melaninogenica	23	23.9
Peptostreptococcus sp. + Prevotella melaninogenica	19	19.8
Chlamydia trachomatis	27	28.1
Ureaplasma urealyticum	12	12.5
Mycoplasma genitalium	17	17.7
N.gonorrhoeae	2	2.1

ciations of obligate anaerobes and *Chlamydia trachomatis* were found in 69 (71.8%) of these patients; 29 (30.2%) – microaerophiles; 14 (14.6%) – the combination of different species of opportunistic pathogens; in 19 (19.8%) – the growth of monocultures of opportunistic pathogens in titers coming to 10x6 -10x8. The growth of monoculture, represented by group B *Streptococcus* was found in 8 (8.3%) cases, *Enterococcus faecalis* – in 12 (12.5%), *Mycoplasma genitalium* – in 17 (17.7%). It is worth noting that obligate anaerobes and *Chlamydia trachomatis* were only found in the uterine cavity as an association.

The microbiota, consisting of viruses and bacteria, was found in patients of the group I when conducting a comprehensive microbiological analysis of the content of the uterine cavity. Whereas, separate opportunistic pathogens were predominant in a culture, in the titers exceeding 10^4 CFU/ml, in the same patient categories in case of culturing.

The analysis of the PCR test enabled to identify associations of various virus strains with *Myco-* and *Ureaplasma*, *Chlamydia* in 23 (23.9%) patients of the group I. The predominance of *Herpes* simplex over CMV infection

Table VII. PCR test of the sample from the uterine cavity in patients of the group I (abs. n., %)

Microorganisms	Group I n=96	
	Abs. n.	%
Trichomonas vaginalis	-	-
Chlamydia trachomatis	28	29.2
Ureaplasma urealyticum	19	19.8
Mycoplasma genitalium	24	25.0
N.gonorrhoeae	2	2.1
Herpes simplex virus I, II	7	7.3
Cytomegalovirus	2	2.1
HRV	-	-

should be noted: 7 (7.3%) and 2 (2.1%), respectively ($p < 0.05$) (Table VII).

DISCUSSION

During analyses of microbiocenosis of vaginal microflora, cervical canal and uterine cavity women with infertility and sexually transmitted diseases in history formed a group with high index of persistent urogenital infection. It was confirmed by a number of international and native authors [2, 3, 12].

Bacterial and viral contamination of endometrium is a consequence of insufficiency of immune system and nonspecific body defense system (complement system, phagocytosis) to eliminate infection factor fully, in order to restrict its spread by activation of T-lymphocytes (T-helpers, natural killers) and macrophages. Furthermore, mononuclear phagocytes, natural killers, T-helpers act in the focus of inflammation and produce different cytokines, that leads to impossibility to form local immunosuppression in the preimplantation period, which is needed in order to form protective barrier and prophylaxes of abruption of a fetus that is a half- heterogenous for mum. [9, 13, 14].

According to a number of authors, the incidence of chronic endometritis in women with infertility is 12,3-60,4%, forming the only cause in 18,8% cases. [3, 5, 6]. According to publications of V.I.Krasnopolski [12], the genital infection, diagnosed by PCR investigation of content of cervical canal, uterine cavity of patients with chronic endometritis includes: chlamydiosis – 14,9%, genital herpes – 33,6%, ureaplasmosis – 37,8%, mycoplasmosis – 11,6%, cytomegaloviral infection – 18,9% of patients. Taking into account the up to date peculiarities of chronic endometritis course, with prevalence of latent forms of diseases, recently the main clinical symptom of chronic endometritis is dysfunction of reproductive function such as infertility, including non-effectiveness of assisted reproductive technologies. [4, 6]. The date collected by a complex analyses of microbiocenosis of vagina, cervical canal and uterine cavity of investigated patients are the same as in the results in literature [16, 17, 18].

It is established, that the duration of microbial agent persistence in endometrium is the main factor, that is defining the stage of tissue damage and the severity of functional disorders of endometrium. The disorder of local resistance mechanisms leads to microbial agents persistence in endometrium, initializing chronical course of inflammatory process. Thus, the above mentioned data indicate the need of antibacterial, antiviral therapy depending on etiological factors in preconceptional period.

CONCLUSIONS

The predominance of the mixed microbiota, represented by various associations of opportunistic and common pathogens, was identified in infertile women with the past history of STIs after culturing samples from the uterine cavity. Chronic endometritis is caused by a persistent viral-bacterial infection in the uterine cavity, which differs in the species composition from the microbiota of the lower genital tract.

REFERENCES

1. Rowley J., Toskin I., Ndowa F. Global incidence and prevalence of selected curable sexually transmitted infections: 2008.
2. World Health Organization (WHO) Library Cataloguing-in-Publication Data Global strategy for the prevention and control of sexually transmitted infections: 2006 – 2015 : breaking the chain of transmission. Geneva; World Health Organization. 2007.
3. Global`naya strategiya sektora zdravookhraneniya po infekcziyam, peredavaemy`m polovy`m putem, na 2016–20gg.: na puti k likvidaczii IPPP. [Global health sector strategy on sexually transmitted infections 2016–2021: Towards ending STIs]. Geneva: WHO. 2016: 60. (In Russian).
4. WHO guidelines for the treatment of Neisseria gonorrhoeae. WHO; 2016. http://apps.who.int/iris/bitstream/10665/246114/1/9789241549691-eng.pdf.
5. Mavrov G.I`., Shherbakova Yu.V., Osi`ns`ka T.V. Novi`tni` metodi strimuvannya i`nfekczi`j, shho peredayut`sya statevim shlyakhom [Novel methods of containment of sexually transmitted infections]. Infectious diseases. 2019;1 (95): 4-10. (in Ukrainian).
6. Bondarenko G. M., Mavrov G. I., Osinska O. et al. Perinatal`na i`nvazi`ya trichomonas vaginalis, yak problema reproduktivnoyi mediczin. [Perinatal invasion of trichomonas vaginalis as a problem of reproductive medicine] Journal of the National Academy of Medical Sciences of Ukraine. 2016;22(3-4): 368-376. (in Ukrainian).

7. Rishhuk S.V., Dushenkova T.A. Optimizacziya diagnostiki reproduktivno znachimy`kh infekczij u polovy`kh par. [Optimization of diagnostics of reproductively significant infections in sexual couples]. Terra Medica. 2016; 4: 20-33.
8. Rishhuk S.V., Vazhbin L. B., Akhunova N. R. et al. Prezentacziya Metodicheskikh rekomendaczij VOZ po khlamidijnoj infekczii. [Presentation of the WHO Guidelines for Chlamydial Infection] Bulletin of the Orenburg Scientific Center of the Ural Branch of the Russian Academy of Sciences. 2014. http://elmag.uran.ru:9673/Numbers/20144/Articles/Rishchuk%20et%20al20144.pdf
9. Rishhuk S.V., Kakhiani E.I., Tatarova N.A. et al. Infekczionno-vospalitel`ny`e zabolevaniya zhenskikh polovy`kh organov: obshhie i chastny`e voprosy` infekczionnogo proczessa: uchebnoe posobie [Infectious and inflammatory diseases of the female genital organs: general and specific issues of the infectious process: a tutorial]. Publishing house of the North-Western State Medical University named after I. I. Mechnikov. 2016: 84.
10. Guffey M. B., Richardson B., Husnik M. et al. Sexually Transmitted Infections. 2014;90(5): 363–369.
11. Unanyan A. L., Snarskaya E. S., Lomonosov K. M. et al. Rol` infekczij, peredavaemy`kh polovy`m putem, v razvitii zhenskogo besplodiya: strategii terapii i profilaktiki [The role of sexually transmitted infections in the development of female infertility: therapy and prevention strategies] Russian Journal of Skin and Venereal Diseases. 2014;5:59-62.

ORCID and contributionship:
Olena I. Krotik: 0000-0002-1677-6634 [A,B,C,DE,F]

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CORRESPONDING AUTHOR
Olena I. Krotik
Shupyk National Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine
9 Dorohozhytska St., 04112 Kyiv, Ukraine
tel: +380 67 804 33 11
e-mail: krotik.elena@gmail.com

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ORIGINAL ARTICLE

SPECTRUM OF CAUSATIVELY RELEVANT AEROALLERGENS OF SOUTHERN UKRAINE WHICH PROVOKE ALLERGIC REACTION IN PATIENTS WITH POLLINOSIS ON THE EXAMPLE OF KHERSON REGION

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Anastasiia V. Myrhorod, Valentyna I. Velychko, Viktoriia Y. Nakhashova
ODESSA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE

ABSTRACT

The aim: the analysis of spectrum of causatively relevant aeroallergens of southern Ukraine which provoke allergic reaction in patients with pollinosis.

Materials and methods: ambulatory medical records data and skin prick-testing results of 477 patients afflicted with pollinosis in Kherson (250 women – 52,41 % and 227 men – 47,59 %) aged from 19 to 66 years old (38,05±0,45) have been analyzed retrospectively.

Results: all patients revealed clinical implications mainly during the September-October period. Clinically 151 patients (31,66%) showed severe and 326 (68,34%) moderate to severe clinical progression. Composite family plants (composites) proved to be the most widespread plants (69,76 %) among all pollen aeroallergens of Kherson region. Weeds and sunflower showed the highest percentage among composites: Ambrosia topped to 79,87 %, Helianthus (sunflower)– 75,68 %, Artemisia – 42,98 %, and Cyclachaena (sump weed) – 36,48 %. Wild and cultivated grains' aeroallergens toped to 28,22 %. Among them the sensibility to Lolium perenne (19,28 %), Festuca pratensis (17,82 %), Dactylis glomerata (17,82 %) has been noticed more often. Tree aeroallergens topped to 2,03 %.

Conclusions: The third wave of pollen formation (September-October period) appeared to be the most significant and clinically hard for Kherson region. According to the specific allergologic research data, allergens of the composite family plants, among which pollen of Ambrosia, Helianthus, Artemisia and Cyclachaena cause more frequent sensibility, turned to be the most widespread causatively relevant aeroallergens in Kherson region which provoke allergic reaction in patients with pollinosis. The second place was taken by wild and cultivated grains' aeroallergens, among which sensibility to Lolium perenne, Festuca pratensis, Dactylis glomerata proved to be the most common.

KEY WORDS: pollinosis, spectrum of aeroallergens, composite family plants, southern Ukraine

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INTRODUCTION

Allergic disorders (AD) are a serious prevalence cause in all world and a significant problem to health care system in general, both for the developed and yet developing countries. At least 30% of the world population and 80% of families are suffering from AD [1].

In recent decades the number of patients with AD nearly doubled, and according to the World Health Organization forecast, in 30 years the majority of Earth population will be suffering from allergy.

For now, one of the most widespread AD is pollinosis. Frequency of its prevalence ranges from 1 to 40% [2, 3]. Pollinosis is characterized by seasonal occurrence, variety of clinical implications, possible polyvalent allergy and food sensibility development, that complicate diagnostics and treatment of this pathology. Pollinosis symptoms are caused by general inflammatory changes of mucous coats, first of all, eyes and airways, and can significantly influence patients' lives. Clinically pollinosis occurs in the form of rhinoallergosis or rhinoconjuctivitis (to 95 % [4]), and atopic asthma.

Pollinosis peculiarities depend on a variety of factors, first of all on climate-geographically and ecologically related ones. Every region has its own distinctive spectrum of allergen plants and pollen formation calendar, in connection to that the course of disease might vary within one country or even region.

Therefore, three main periods of pollen formation are relevant for our country: spring period (April-May), summer period (June-July) and summer-autumn period (July-September). Also three main groups of plants-allergens are singled out: trees, grains and composites, the majority of which are weeds. For the first (spring) period pollen formation of trees is typical (birch, oak, alder, linden, ash, poplar, elm, acacia, willow and others). The second (summer) period is characterized by pollen formation in wild and cultivated grains (rye, corn, Poa pratensis, Phleum pratense, Festuca pratensis, Dactylis glomerata, wheat-grass (Agropyron) , Bromus, Lolium perenne and others). Considering the third (summer-autumn) period, pollen formation in composites (Ambrosia, Artemisia, Chenopodium, Helianthus, Cyclachaena and others) is common.

At the present moment there exist some scientific works on spectrum of aeroallergens study for certain regions. For instance, aerobiological researches distinguishing in

Table I. The results of specific allergologic examination by method of skin prick-testing with pollen allergens in patients with pollinosis of Kherson town, number of patients n=477

Trees		Grains		Composites	
Allergen	**Abs. number (%)**	**Allergen**	**Abs. number (%)**	**Allergen**	**Abs. number (%)**
Acacia	9 (1,89)	Dactylis glomerata	85 (17,82)	Ambrosia	381 (79,87)
Walnut	14 (2,94)	Rye	56 (11,74)	Chenopodium	28 (5,87)
European alder	0	Alopecurus pratensis	16 (3,35)	Artemisia	205 (42,98)
Linden	5 (1,05)	Festuca pratensis	85 (17,82)	Cyclachaena	174 (36,48)
Poplar	5 (1,05)	Zea mays (corn)	55 (11,53)	Helianthus	361 (75,68)
Birch	0	Lolium perenne	92 (19,28)	Dandelion	19 (3,98)
Oak	0	Agropyron repens	33 (6,92)	__	___
European hornbeam	0	Bromus erectus	3 (0,62)	__	___
Chestnut	0	Phleum pratense	34 (7,13)	___	___
Sugar maple	0	Poa pratensis	6 (1,26)	___	___

Table II. Classification of pollen allergens revealed in patients examined according to pollen allergens groups

Pollen Allergens Group	Trees, n (%)	Grains, n (%)	Composites, n (%)
Total number of positive results n=1646	33 (2,03)	465 (28,23)	1149 (69,76)

methods and time have been carried out in many cities such as Kyiv, Vinnytsia, Zaporizhzhia, Lviv, Odesa, Poltava, Donetsk, Dnipro and Simferopil. Aeropolynological monitoring procedures are being constantly held in Vinnytsia, Lviv, Zaporizhzhia [5].

But despite this, there is relatively little information regarding spectrum of aeroallergens of southern Ukraine among patients afflicted with pollinosis. Moreover, no similar research was carried out in Kherson region before. Thus, our aim was to explore spectrum of causatively relevant aeroallergens analyzing the results of specific allergologic examination based on skin prick-testing method among patients afflicted with pollinosis in Kherson.

THE AIM

The aim was the analysis of spectrum of causatively relevant aeroallergens of southern Ukraine which provoke allergic reaction in patients with pollinosis.

MATERIALS AND METHODS

In hindsight, we researched the data of ambulatory medical records and results of skin testing with allergens of 477 patients who applied to town allergology centre based on Kherson Town Clinical Hospital n.a. Ye.Ye. Karabelesh during the period of 2012 to 2019 years. A specific allergologic examination has been carried out by the method of skin prick-testing with allergens of Ukrainian production (Vinnytsia, LLC "Imunolog"). Established allergens of trees, composite family plants and grains, as well as domestic allergens (acarids Dermatophagoides farinae and Dermatophagoides pteronyssinus, pillow feathers, epidermal agents) were used. Patients' examination was done during the remission period (the end of October-April). Entry criteria resulted in making pollinosis diagnosis in patients above 18 years old. Entry criteria: infant age, negative results of skin prick-testing. Statistic data estimation was performed with the help of Microsoft Office Excel 2010.

RESULTS

In the whole, ambulatory medical records and the results of skin prick-testing with of 477 patients: 250 women (52,41 %) and 227 men (47,59 %) aged from 19 to 66 years old.

The average age of examined patients was 38,05±0,45.

All the examined patients had symptoms of pollinosis in anamnesis and revealed clinical implications mainly during the period from August to September.

Monovalent allergy, in other words sensibility to only one aeroallergen, was discovered only in 7,59 % patients (36 examined). The majority of patients had a polyvalent sensibility to more than one aeroallergen – 92,4 % (441 patient). Among them 106 patients (22,22 %) except sensibility to pollen aeroallergens, had also sensibility to domestic and/or epidermal ones (i. e. had a secondary diagnosis of annual rhinoallergosis).

Clinically, 151 patients (31,66%) had severe and 326 (68,34%) moderate to severe disease progression.

The results are included in the tables 1, 2.

Among pollen aeroallergens of Kherson region, the most widespread ones are composites (69,76 %).

Among composites the greatest percentage showed weeds and sunflower: Ambrosia – 79,87 %, Helianthus (sunflower) – 75,68 %, Artemisia – 42,98 %, and Cyclachaena (sump weed) – 36,48 %. Aeroallergens of wild

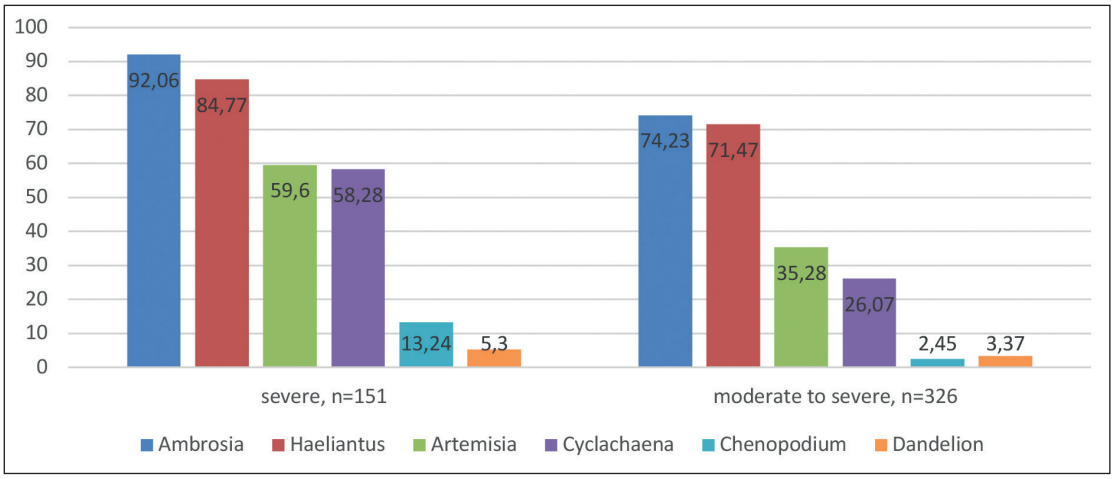

Fig. 1. Positive results of skin prick-testing compared to composites' allergens in patients with severe and moderate to severe clinical progression.

and cultivated corn plants were estimated in 28,22 %. The most frequent sensibility among them was noticed to Lolium perenne (19,28 %), Festuca pratensis (17,82 %), Dactylis glomerata (17,82 %). Aeroallergens of trees registered only 2,03 %.

DISCUSSION

The average age of examined patients was 38,05±0,45 that confirms data of dominating disease incidence among people of young, able to work age [6].

The most widespread pollen aeroallergens of Kherson region are composites (69,76 %), that proves the similarity of spectrum of causatively relevant aeroallergens with other region of southern Ukraine, particularly Odesa, Zaporizhzhia, Dnipro regions and Temporarily Occupied Territory of Crimea [7, 8, 9, 10]. However, received data greatly differ from the spectrum of other regions of our country (for example, in Vinnytsia and Lviv regions trees took the first place, in Kyiv region – grains [4, 11, 12].

One might assume that received relatively large amount of positive results of skin prick-testing with composites' allergens comparing to trees' and grains' allergens is connected to the fact that allergens of the first ones are the most widespread and aggressive in our region. It is also proved by that the majority of patients with severe clinical progression revealed positive skin prick-testing results with composites' aeroallergens comparing to the patients with moderate to severe clinical progression (Pic.1). Number of pollen allergens, before which the results had been positive, also proves the complexity. Among the patients with severe clinical progression this number was estimated in 5±0,17 and appeared to be credibly higher ($p \leq 0,01$) than among the patients with moderate to severe clinical progression (2,7±0,08).

CONCLUSIONS

1. For Kherson region the third wave of pollen formation is the most typical and clinically hard (August-September period) – almost all patients examined reveal exacerbation during the indicated period and positive results of skin prick-testing with plants-allergens producing pollen in this time period.
2. The most widespread causatively relevant aeroallergens in Kherson region in patients with pollinosis according to the data of specific allergologic research proved to be allergens of composites (69,76 %), among which the most frequent is sensibility to pollen of Ambrosia, Helianthus, Artemisia and Cyclachaena.
3. The second place is taken by aeroallergens of wild and cultivated grains (28,23 %), among which the most frequent is sensibility to pollen of Lolium perenne, Festuca pratensis, Dactylis glomerata.
4. The complexity of clinical progression in patients afflicted with pollinosis is caused by number of pollen allergens sensibility proved (difference is credibly higher in patients with severe clinical progression comparing to moderate to severe one ($p \leq 0,01$)).
5. The subject of spectrum of causatively relevant aeroallergens investigation and analysis of southern Ukraine, particularly Kherson region, claims further research. The result that is to be expected is pollinosis diagnostics and treatment optimization and prevention of food sensibility development in such patients considering regional peculiarities of pollen formation in plants-allergens.

REFERENCES

1. Sanchez-Borges M., Martin B.L., Muraro A.M. et al. The importance of allergic disease in public health: an iCAALL statement. World Allergy Organization Journal. 2018/ https://doi.org/10.1186/s40413-018-0187-2.
2. Pukhlyk B.M., Pollinos [Pollinosis]. Monography.2017; 60. (In Russian).
3. Horiachkina L.A., Drobik O.S., Nasunova A.Yu. Pollinosy: sovremenniy vzgliad na problem. [Pollinosis diseases: contemporary look at the problem]. Newsletter of Family Medicine. 2012; (1):10-16. (In Russian).
4. Klymenko V.A. Regionarni osoblyvosti pylkovoi sensybilizatsii (za danymy Oblasnogo dytiachogo alergotsentru). [Regional peculiarities of pollen sensibility (in accordance with the data of Regional child allergocentre]. Clinical immunology. Allergology. Infectology. Special issue: Allergic diseases in clinical practice. 2012; 66-67. (In Ukrainian).

5. Rodinkova V.V. Osnovnyi aeropalinologichnyi spektr mist tsentralnoi, pivdennoi ta shidnoi Ukrainy. [Basic aeropalinologic spectrum of cities in central, southern and eastern Ukraine]. Biology and medicine achievements. 2013;(2):11-15. (In Ukrainian).
6. Brożek J.L., Bousquet J., Agache I. et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2016. Allergy Clin Immunol. 2017;140(4):950-958.
7. Dytiatkovska Ye. M. Osoblyvosti sensybilisatsii u khvorykh na polinos v m. Dnipropetrovsku. [Sensibility peculiarities in patients afflicted with pollinosis in Dnipropetrovsk city]. Rhinology. 2011; 2: 17-20. (In Ukrainian).
8. Tolstanov A.K., Harmider O.V., Hozhenko A.I. Osobennosti zabolevaemosti i klinicheskaia kharakteristika bolnykh pollinozom v g.Odessa. [Disease incidence peculiarities and clinical characteristics of patients afflicted with pollinosis in Odessa city]. Topical issues of transport medicine. 2013;4(34):60-68.(In Russian).
9. Malieieva H.Yu., Prykhodko O.B. Dynamika palinatsii ambrozii v Zaporizhzhi v period iz 2012 po 2016 rik. [Palination dynamics of ambrosia in Zaporizhzhia during 2012-2016 period]. Newsletter of Zaporizhzhia National University. 2016;2:121-129. (In Ukrainian).
10. Znamenska L.K. Spektr prichinnoznachimykh allergenov, vyzyvaiushchikh pollinoz u zhytelei Simferopolia. [Spectrum of causatively relevant aeroallergens which provoke pollinosis in citizens of Simferopol]. Tavrichesk medicine-biological newsletter. 2013;4(64):60-64. (In Russian).
11. Besh L.V., Novikevych S.Z., Zadvorna O.I. et al. Dynamika struktury sensybilizatsii do pylku sered dytiachogo naselennia Lvivskoi oblasti protiagom 20-richnogo sposterezhennia. [Dynamics of structure of sensibility to pollen among infant population of Lviv region during 20 years observation period]. Child's health. 2014;7(58):37-42. (In Ukrainian).
12. Zaikov S.V., Hatska D.O., Korytska I.V. Spektr sensybilizatsii do allergeniv u ditei ta molodi Vinnytskoi oblasti z respiratornoiu alergiieiu. [Spectrum of sensibility to allergens among children and youth with respiratory allergy in Vinnytsia region]. Asthma and allergy. 2016;4:25-28. (In Ukrainian).

ORCID and contributionship:
Anastasiia V. Myrhorod: 0000-0001-8094-5693 [A,B,C,D,E]
Valentyna I. Velychko: 0000-0003-3607-0265 [A]
Viktoriia Y. Nakhashova: 0000-0003-1891-6984 [F]

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CORRESPONDING AUTHOR
Anastasiia V. Myrhorod
Odessa National Medical University
2 Valykhovskyi prov., 65000 Odesa, Ukraine
tel: +380991572250
e-mail: nastya.krysenko1404@gmail.com

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ORIGINAL ARTICLE

OPTIMIZATION OF LOCAL ANESTHESIA OF DENTAL PATIENTS DURING CYSTECTOMIES OF RADICULAR CYSTS THAT HAVE GROWN INTO THE MAXILLARY SINUS OR NASAL CAVITY

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Oleg Ya. Mokryk[1], Davyd S. Avetikov[2], Ivan S. Sorokivskyi[1], Solomiya T. Havryltsiv[1], Nataliia M. Sorokivska[1]
[1]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE
[2]UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY, POLTAVA, UKRAINE

ABSTRACT

The aim: To provide clinical evaluation of the effectiveness of anesthesia for cystectomy of radicular cysts that have grown into the maxillary si-nus or nasal cavity using different techniques of endonasal block-ade of the nasopalatine nerve.

Materials and methods: Clinical observations were conducted on 52 patients of different age and sex. These patients were diagnosed with large radicular cysts (more than 3.0 cm in diameter) that had grown into the maxillary sinus (33 cases) or into the nasal cavity (19 cases). The surgical excisions of cysts (oroantral cystectomies) were performed un-der local potentiated anesthesia. Patients were divided into two clinical groups, depending on the methods used for endonasal blockade of the nasopalatine nerve: the main and the control group. Patients in the main group underwent endonasal blockade of the nasopalatine nerve at the point of its emergence from the pterygopalatine ganglion. In the control group, the neural blockade was performed at the entry of this nerve into the incisive canal.

Pain sensitivity and perception in patients were studied using subjective and objective methods. The data was analyzed by means of the Pearson's chi – square tests.

Results: During the enucleation of radicular cysts that grew into the inferior nasal meatus and maxillary sinus in patients of the main group there was no pain observed. There were no manifestations of pain-induced stress neither from the side of autonomic system, nor physical or emotional manifestations of pain, which confirmed the effectiveness of nasopalatine nerve anesthesia. In patients of the control group during similar operation the full effect of local anesthesia was achieved in 56.0% of cases ($\chi 2 - 9{,}270$, $p = 0{,}003$). The rest of the patients during cystectomies showed some signs of pain-induced stress from the side of autonomic system or in the form of physical or emotional manifestations.

Conclusions: Endonasal blockade of the nasopalatine nerve at the point of its emergence from the pterygopalatine ganglion invariably provided a complete analgesia during enucleation of radicular cysts that have grown into the maxillary sinus or nasal cavity. Anesthesia of the nasopalatine nerve at its entry into the incisive canal allows pain-less cystectomy of radicular cysts that have spread to the front of the inferior nasal meatus from the front teeth.

KEY WORDS: radicular cyst, cystectomy, maxillary sinus, nasal cavity, nasopala-tine nerve, local anesthesia, pain-induced stress

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INTRODUCTION

Dental surgeries may cause pain and lead to pain-induced stress reactions [1 – 3]. Among the factors that determine their development are psycho-emotional tension (fear, anxiety, phobias), pain, pathological reflexes and other factors [4 – 8]. High-quality anesthesiological support allows to provide anti-stress protection of surgical patients. Conductive anesthesia of peripheral branches of the trigeminal nerve (trigeminal nerve block) is most commonly used for anesthesia of the tooth – alveolar complex according to standard methods [1, 2, 6, 9, 10]. Surgical interventions on the maxillary sinus in the treatment of chronic odontogenic sinusitis and during cystectomies of odontogenic cysts that have grown into the maxillary sinus can be performed either under general anesthesia [11 -13] or under local potentiated anesthesia [14, 15]. In the latter case, a desirable analgesic effect is achieved by a combination of premedication (analgesia) and local anesthesia of the sub-orbital and great palatine nerves, the upper posterior alveolar branches of the maxillary nerve. In addition, when performing surgical manipulations in the nasal cavity – endoscopy of the maxillary sinus, enucleation of the odontogenic cysts that have grown into the inferior nasal meatus, it is also necessary to block the nasopalatine nerve during anesthesia of the operation site [16 – 19]. According to some authors [20], the most optimal is the nerve block injection of the pterygopalatine ganglion (ganglion pterygopalatinus), approaching through the greater palatine canal (canalis palatinus major). We believe that this method of the pterygopalatine ganglion blockade is not always easy to perform due to the anatomical variability of the greater palatine canal [21 – 23], in-stead it is possible to use in such cases endonasal methods of an-esthesia of the nasopalatine nerve at the point of its emergence from the pterygopalatine ganglion or at its entry into the incisive canal.

Table I. The Sounds, Eyes, and Motor (SEM) scale for measuring the comfort or discomfort

Observations	Comfort	Discomfort		
		Mild pain	Moderate pain	Severe pain
Sounds	No sounds indicating pain	Nonspecific sounds; possible pain indication	Specific verbal complaints, e.g., "Ouch", raises Voice	Verbal complaint indicates intense pain, e.g., scream, sobbing
Eyes	No eye signs indicating discomfort	Eyes wide, show of concern, no tears	Watery eyes, eyes flinching	Crying, tears running down face
Motor	Hands relaxed; no apparent body tension	Hands show some distress or tension, grasps chair due to discomfort, muscular tension	Random movement of arms or body without aggressive intention of physical contact, grimace, twitch	Movement of hands to make aggressive physical contact, pulling head away

THE AIM

To provide clinical evaluation of the effectiveness of anesthesia for cystectomies of radicular cysts that have grown into the maxillary sinus or nasal cavity using different techniques of endonasal blockade of the nasopalatine nerve.

MATERIALS AND METHODS

Clinical observations were conducted on 52 patients of different ages (from 20 to 65) and sex (34 males and 18 females). These were the patients who received treatment at the department of surgical dentistry and maxillofacial surgery. All of them were diagnosed with radicular cysts of large size (> 3.0 cm), which penetrated the maxillary sinus (33 cases) or the nasal cavity – 19 cases. Cystectomies (oroantral cystectomy) were performed under local potentiated anesthesia. All patients received pre-operational premedication (intramuscular): 2% solution of promedol (trimeperidine) – 1 ml, 0.5% solution of sibazone (diazepam) – 2.0 ml, 1% solution of diphenhydramine (diphenhydramine hydrochloride) – 1, 0 ml, 0.1% solution of atropine – 1.0 ml. For conducting of local an-esthesia (infraorbital, palatal, tuberal) we used "Ultracain® D-S forte" (articaine hydrochloride and epinephrine solution, 1:100000 dilution).

The patients were divided into two clinical groups, depending on the methods used for endonasal blockade of the nasopalatine nerve: the main and the control group. The patients in the main group (27 people) had a blockade of the nasopalatine nerve by application anesthesia of the nasal mucosa near the posterior edge of the middle nasal meatus, at the point of the nasopalatine nerve emergence from the pterygopalatine ganglion [24]. To do this, a nasal probe with a sterile cotton swab soaked in 10% solution of lidocaine with adrenaline in 1: 100 000 dilution, was inserted into the dorsum of the nasal cavity and at an angle of 25 ° – 30 ° to the hard palate was advanced in the middle nasal passage to a depth of 4.5 – 5, 0 cm. Our choice of such technical parameters was based on the findings of morphometric studies obtained by the authors [25].

In 25 patients of the control group, standard intranasal blockade of the nasopalatine nerve was performed by application anesthesia with 10% lidocaine solution with adrenaline of the mucous membrane at the base of the nasal septum, in the funnel-shaped area where this nerve enters the incisive canal [26].

Pain sensitivity and perception in patients were studied using subjective and objective methods. Pain sensitivity was determined by injection of a needle (pinprick) into the mucous membrane. The assessment of pain sensitivity was performed on a four-point scale in each quadrant: 0 points – no sensitivity, 1 point – sensitivity is sharply reduced, 2 points – sensitivity is moderately reduced, 3 points – tactile and pain sensitivity is completely preserved [27]. Pain perception during local anesthesia administration was evaluated by the Sounds, Eyes and Motor (SEM) scale [28]. SEM scale was used to assess the observed pain. It is divided into two categories of comfort and discomfort. The discomfort response is further divided into three subscales: mild pain, moderate pain and severe pain (Table I).

Autonomic reaction of the cardiovascular system to pain was evaluated by the perfusion index (PI) monitored with the pulse oximeter Elera SH-K3 (Hong Kong). The normal PI range is 5–6 %. It de-creases with the narrowing of the lumen of the peripheral blood vessels caused by the pain triggers [29].

The protocol used in this study conformed to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Danylo Halytsky Lviv National Medical University and of the Ukrainian Medical Stomatological Academy.

The reliability of the obtained results was evaluated according to the reliability criterion of the Student by statistically computing the data using the commonly used methods of variation statistics from the package «Statistica-8». The data was analyzed by means of the Pearson's chi–square tests.

RESULTS

During the enucleation of the radicular cysts that have penetrated the inferior nasal meatus, all patients in the main group had no pain. According to the prick-test, all of them revealed a complete loss of pain sensitivity (0 points) of the mucous membrane of the inferior nasal meatus in the area of localization of radicular cysts, which confirmed the effectiveness of the applied method of anesthesia of the nasopalatine nerve. While in the group, where we per-formed the blockade of peripheral branches of the maxillary nerve (suborbital, greater palatine, nasopalatine nerves, upper posterior alveolar branches) the outcome was different. In

Table II. The clinical effectiveness of used intranasal methods of anesthesia of the nasopalatine nerve

Operation site of cystectomy of radicular cyst	Method of anesthesia of the nasopalatine nerve near the incisive canal (n = 25)		Method of anesthesia of the nasopalatine nerve near the ganglion pterygopalatinus (n = 27)		Pearson's coefficient χ2 (p)
	Total anesthesia in the operating area	Partial anesthesia in the operating area	Total anesthesia in the operating area	Partial anesthesia in the operating area	
Cystectomy in the maxillary sinus	9 cases	7 cases	15 cases	2 cases	4,251 (p = 0,040)
Cystectomy in the nasal cavity	5 cases	4 cases	10 cases	no cases	5,630 (p = 0,018)
Totally n - cases (%)	14 cases (56,0 %)	11 cases (44,0 %)	25 cases (92,6 %)	2 cases (7,4 %)	9,270 (p = 0,003)

15 cases we also managed to painlessly perform oroantral cystectomy and removed pathologically altered (hyperplastic) mucosa, pathological granulations. However, 2 patients of this clinical group at the stage of formation and mobilization of the buccal muco-periosteal flap for plastic closure of oroantral communications, formed in the sockets of the removed third upper molars, demonstrated emotional/motor (mild pain – beyond the SEM scale) and autonomic (index RI – 3.5 – 4.0%) manifestations of the pain-induced stress. The prick test revealed moderate pain sensitivity (2 points) of the mucous mem-brane in the distal (buccal) part of the mucoperiosteal flap, which is associated with possible participation of the buccal nerve in the sensitive innervation of this anatomical area of the oral cavity [30]. After additional infiltration anesthesia, we finally achieved a complete loss of pain sensitivity in this area.

The patients of the control group during cystectomy of radicular cysts that have penetrated the nasal cavity experienced and demonstrated pain more often than the patients of the main group (χ^2 – 5,630,p = 0.018), which was associated with insufficient effectiveness of local anesthesia of the surgical site. In particular, 4 patients retained pain sensitivity during enucleation of odontogenic cyst, which spread from the periapical areas of the "causal" premolars to the maxillary sinus and the distal direction of the lower nasal passage.

The pain sensitivity in the mucous membrane of the bottom of the nasal cavity was evaluated in 2.7 ± 0.4 points. On the doctor's at-tempt to begin the surgery, such patients manifested motor (dis-comfort according to SEM scale) and autonomic (PI = 2.9 ± 0.6%) reactions to pain triggers. After additional infiltration anesthesia the total loss of sensitivity was reached. It should be noted that in 5 patients whose radicular cysts were located at the level of the roots of the front teeth of the upper jaw and grew into the frontal part of the nasal cavity, cystectomy operations were painless.

During oroantral cystectomy, pain occurred in 6 patients at the stage of removal of pathologically altered mucous tissue on the medial (nasal) septum of the maxillary sinuses. Its distal part, at the level of the middle nasal meatus, turned out to be especially "sensitive". This testified to the ineffectiveness in such cases of the classical intranasal technique of incisal anesthesia. In one case, we observed insufficient local anesthesia in the area of the bone defect of the third molar socket during its plastic closure with a mucoperiosteal flap. All the above-mentioned patients experienced emotional discomfort due to pain, there was a decrease in the per-fusion index. Intergroup differences in the effectiveness of anesthesia support for cystectomy operations performed in the maxillary sinuses were statistically significant – χ^2 – 4,251, p = 0,040 (Table II).

DISCUSSION

On the background of application anesthesia of the nasal mucosa, which is innervated by the nasopalatine nerve [31], diagnostic and therapeutic procedures are performed – endoscopy of the paranasal sinuses, tamponade of the nasal cavity [32, 33]. Electroneurophysiological studies have shown that in the innervation of the pulp of the incisors of the maxilla in addition to the anterior alveolar branches of the infraorbital nerve, nasopalatine nerve may also be involved. When it is blocked in the lower nasal passage before entering the incisal canal, the effect of anesthesia of the pulp of these teeth is potentiated [34]. To increase the effectiveness of analgesia in this group of teeth, it is advisable to inject a local anesthetic in the middle nasal passage [35].

After inserting a cotton applicator impregnated with anesthetic into the middle nasal passage and its advance to the posterior edge of the middle nasal cavity, it is possible to reach the place where pterygopalatinum ganglion (PPG) is located. Its post-synaptic fibers innervate the lacrimal and nasal glands. This autonomic node is involved in the pathogenesis (regulation of vascular response) in various pain syndromes: headache (migraine), trigeminal neuralgia, facial pain induced by herpes virus infection. Clinical studies have shown that these pain disorders can be effectively managed with endonasal blockade of PPG [36 – 39]. In maxillofacial surgery, PPG regional anesthesia is performed mainly by injection, by inserting a needle into the pterygopalatine fossa by extrazygomatic [40, 41], subtemporal [42], palatal [43, 44] accesses.

However, during using of these techniques of conductive anesthesia PPG there is a risk of blood vessels and nerves damage by the injection needle during its advancement , it may break. With excessive administration of a local anesthetic, it can spread through the lower orbital fissure into the orbit and cause diplopia [45 – 48]. Therefore, in our opinion, the safest (the least traumatic) and available for technical performance, free of possible local complications is the endonasal method of PPG blockade, which also blocks the nasopalatine nerve.

CONCLUSIONS

Endonasal blockade of the nasopalatine nerve at the point of its emergence from the pterygopalatine ganglion invariably provides a complete analgesia during enucleation of radicular cysts that have penetrated the maxillary sinus or nasal cavity. An-esthesia of the nasopalatine nerve at its entry into the incisive canal allows painless cystectomy of radicular cysts that have spread to the front of the inferior nasal meatus from the front teeth.

REFERENCES

1. Gadve V.R., Shenoi R., Vats V., Shrivastava A. Evaluation of anxiety, pain, and hemodynamic changes during surgical removal of lower third molar under local anesthesia. Ann Maxillofac Surg. 2018;8:247-53.
2. Sharma A., Pant R., Priyadarshi S. et al. Cardiovascular changes due to dental anxiety during local anesthesia injection for extraction. J Maxillofac Oral Surg. 2019;18(1):80-7.
3. Tarazona-Álvarez P., Pellicer-Chover H., Tarazona-Álvarez B. et al. Hemodynamic variations and anxiety during the surgical extraction of impacted lower third molars. J Clin Exp Dent. 2019;11(1):27-32.
4. Pani S.C., Al Garni B., Al Zain L.M., Al Qahtani N.S. Assessment of the impact of stress and anxiety on pain perception in patients undergoing surgery for placement of their first dental implant. Oral Health Dent Manag. 2014;13(2):464-8.
5. López-Jornet P., Camacho-Alonso F., Sanchez-Siles M. Assessment of general pre and postoperative anxiety in patients undergoing tooth extraction: A prospective study. Br J Oral Maxillofac Surg. 2014;52:18-23.
6. Araújo R.Z., Pintor Junior A.A.C., Sigua-Rodriguez E.A. et al. Pain control in third molar surgery. Int. J. Odontostomat. 2016; 10(3):385-391.
7. Eroglu C.N., Ataoğlu H., Küçük K. Factors affecting anxiety-fear of surgical procedures in dentistry. Niger J Clin Pract. 2017;20:409-14.
8. Mufti N., Mufti S., Mufti K. Stress and Anxiety in Patients undergoing Dental Extraction. Int J Oral Care Res. 2017;5(1):75-7.
9. Kanakaraj M., Shanmugasundaram N., Chandramohan M. et al. Regional anesthesia in faciomaxillary and oral surgery. J Pharm Bioallied Sci. 2012; 4(2): 264 – 9.
10. Vargas B.A., Astorga P., Rioseco T. Anatomy applied to block anesthesia for maxillofacial surgery. Dental anatomy, Bağdagül Helvacioğlu Kivanç, IntechOpen. 2017.DOI: 10.5772/intechopen.69545.
11. Velasco I., Vahdani S., Nuñez N., Ramos H. Large recurrent radicular cyst in maxillary sinus: A case report. Int. J. Odontostomat. 2017; 11(1):101- 5.
12. Chybicki D., Lipczyńska-Lewandowska M., Ratajek-Gruda M., Janas-Naze A. Massive Radicular Cyst in the Maxillary Sinus as a Result of Deciduous Molar Tooth Pulp Necrosis. Hindawi Case Reports in Dentistry. 2020; 5.
13. Nilesh K., Dadhich A. Unusually large radicular cyst presenting in the maxillary sinus. BMJ Case Rep. 2020;13:e236582.
14. Shamil E., Rouhani M.J., Basetti S. et al. Role of local anaesthetic nerve block in endoscopic sinus surgery: A systematic review and meta-analysis. Clin Otolaryngol. 2018 Oct;43(5):1201- 8.
15. Cavarra F., Tricarico G., Tepedino M. et al. Voluminous maxillary radicular cysts removal under local anesthesia. Minerva Stomatologica. 2019; 68(4):213 – 6.
16. Decker J.R., Dutton J.M. Local anesthesia for nasal and sinus surgery. Ear Nose Throat J. 2013; 92(3):107 – 8.
17. Shamil E., Casselden E., Bast F. et al. Role of local anaesthetic nerve block in patients undergoing endonasal surgery – our experience of 48 patients. Rhinology Online. 2018; 1: 90 – 3.
18. Scott G.M., Diamond Ch., Micomonaco D.C. Assessment of a lateral nasal wall block technique for endoscopic sinus surgery under local anesthesia. Am J. Rhinol Allergy. 2018;32(4):318 -322.
19. Shamil E., Casselden E., Bast F. et al. Role of local anaesthetic nerve block in patients undergoing endonasal surgery – our experience of 48 patients. Rhinology Online. 2018; 1: 90 – 3.
20. Hu Y.K., Yang C., Xu G.Zh., Xie Q.Y. Digital design of functional surgery for odontogenic cyst intruding into maxillary sinus. Brazilian Journal of Otorhinolaryngology. 2018; 84 (2): 212- 9.
21. Soto R.A., Cáceres F., Vera C. Affiliations expand morphometry of the greater palatal canal in adult skulls. J Craniofac Surg. 2015;26(5):1697-9.
22. Bahşi İ., Orhan M., Kervancıoğlu P., Yalçın E.D. Morphometric evaluation and clinical implications of the greater palatine foramen, greater palatine canal and pterygopalatine fossa on CBCT images and review of literature. Surgical and Radiologic Anatomy. 2019; 41(5):551-567.
23. Onurcem D., Varun K., Emel Tuğba A-D. et al. Radio-Morphometric Evaluation of Greater Palatine Canal and Pterygopalatine Fossa Component: Maxillary Anesthetic Implications. Journal of Craniofacial Surgery. 2019; 30 (3): 863 – 7.
24. Malinowski M.N., Bremer N.J. Sphenopalatine Ganglion Block. Deer's Treatment of Pain. Switzerland: Springer Cham. Sphenopalatine Ganglion Block. 2019: 531-5. https://doi.org/10.1007/978-3-030-12281-2_64.
25. Sir E., Eksert S. Morphological Description and Clinical Implication of Sphenopalatine Foramen for Accurate Transnasal Sphenopalatine Ganglion Block: An Anatomical Study. Medeni Med J. 2019; 34(3): 239–243.
26. Shedyakova N.V., Petrikas A.Z.H., Chestnyh E.V. Analgesic preparation of the oral cavity to injection. Institute of stomatology. 2017; 4: 76 – 7.
27. Smith M.H., Lung K.E.. Nerve Injuries after Dental Injection: A Review of the Literature. J. Can Dent Assoc. 2006; 72(6):559–64.
28. Wright G.Z., Weinberger S.J., Marti R., Plotzke O. The effectiveness of infiltration anesthesia in the mandibular primary molar region. Pediatr Dent. 1991;13:278–83.
29. Raouf Mohameda S.A., Mohameda N.N., Rashwanb D. Pulse co-oximetry perfusion index as a tool for acute postoperative pain assessment and its correlation to visual analogue pain score. Research and Opinion in Anesthesia & Intensive Care. 2015; 2(3): 62– 7.
30. Takezawa K., Ghabriel M., Townsend G. The course and distribution of the buccal nerve: clinical relevance in dentistry. Australian Dental Journal. 2018; 63 (1): 66 – 71.
31. Sai Pavithra R., Thenmozhi M.S. Maxillary Nerve Variations and Its Clinical Significance. J. Pharm. Sci. & Res. 2014; 6(4): 203- 5.
32. Balasubramanian T. Local Anaesthesia of Nose and Nasal cavity- A Review. Glob J Oto. 2017; 4(4): 555643.
33. Shamil E., Rouhani M.J., Basetti S. et al. Role of local anaesthetic nerve block in endoscopic sinus surgery: A systematic review and meta-analysis. Clin Otolaryngol. 2018;43(5):1201-1208.

34. Shedyakova N.V., Petrikas A.Z.H., Chestnyh E.V. Analgesic preparation of the oral cavity to injection. Institute of stomatology. 2017; 4: 76 – 7.
35. Menon G.R., Raj J. D.. Intranasal anesthetic spray for maxillary anterior teeth. Drug Invention Today. 2018; 10(2): 3030 – 4.
36. Piagkou M., Demesticha T., Troupis T. et al. The pterygopalatine ganglion and its role in various pain syndromes: from anatomy to clinical practice. Pain Pract. 2012;12(5):399-412.
37. Candido K.D., Massey S. T., Sauer R., et al. A Novel Revision to the Classical Transnasal Topical Sphenopalatine Ganglion Block for the Treatment of Headache and Facial Pain. Pain Physician. 2013; 16:E769-E778.
38. Iwanaga J., Wilson Ch., Simonds E., Vetter M. Clinical Anatomy of Blockade of the Pterygopalatine Ganglion: Literature Review and Pictorial Tour Using Cadaveric Images. The Kurume Medical Journal. 2018; 65(1): 1-5.
39. Cady R., Saper J., Dexter K., Manley H.R. A double-blind, placebo-controlled study of repetitive transnasal sphenopalatine ganglion blockade with tx360® as acute treatment for chronic migraine. 2015;55(1):101-16.
40. Cometa M.A., Zasimovich Y., Smith C.R. Percutaneous sphenopalatine ganglion block: an alternative to the transnasal approach. Int J Obstet Anesth.. 2020; (20):30130-8.
41. Chiono J., Raux O., Bringuier S. et al. Bilateral Suprazygomatic Maxillary Nerve Block for Cleft Palate Repair in Children. Anesthesiology. 2014; 120:1362-9.
42. Oleshchenko I.G., Iureva T.N., Zabolotskii D.V., Gorbachev V.I. Blockade of the pterygopalatine ganglion as a component of combined anesthesia during surgery for congenital cataract. Regionarnaya anesteziya i lechenie ostroy boli (Regional Anesthesia and Acute Pain Management, Russian journal). 2017; 11 (3): 202–207. (In Russian).
43. Borodulin V.G. Maxillary nerve and sphenopalatine ganglion blockade via palatal approach. Folia Otorhinoiaryng et Pathol Respiratoriae. 2014; 20 (2): 28.
44. Hu Y.K., Yang Ch., Xu G.Zh., Xie Q.Y. Digital design of functional surgery for odontogenic cyst intruding into maxillary sinus. Brazilian Journal of Otorhinolaryngology. 2018; 84 (2): 212- 9.
45. Piagkou M., Demesticha T., Troupis T. et al. The pterygopalatine ganglion and its role in various pain syndromes: from anatomy to clinical practice. Pain Pract. 2012;12(5):399-412.
46. Ho K.W.D., Przkora R., Kumar S. Sphenopalatine ganglion: block, radiofrequency ablation and neurostimulation – a systematic review. J Headache Pain. 2017;18(1):118.
47. Boynes S.G., Echeverria Z. Ocular Complications Associated with Local Anesthesia Administration in Dentistry. Dental clinics of North America. 2010;54(4):677-86.
48. Ravi P., Gopi G., Shanmugasundaram S., Raja K.K. Ocular complications with dental local anaesthesia – a systematic review of literature and case report. SADJ. 2015; 70 (8): 354 – 7.

ORCID and contributionship:
Oleg Ya. Mokryk: 0000-0002-4237-3812 [A, E]
Davyd S. Avetikov: 0000-0002-7055-3589 [B]
Ivan S. Sorokivskyi: 0000-0001-5998-6706 [C]
Solomiya T. Havryltsiv: 0000-0002-0528-3607 [D]
Nataliia M. Sorokivska: 0000-0003-3459-8859 [F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Oleg Ya. Mokryk
Danylo Halytsky Lviv National Medical University
69 Pekarska str., 79010 Lviv, Ukraine
tel: +380677698176
e-mail: zahn2008@ukr.net

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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

ULTRAMICROSCOPIC ERYTHROCYTES PROFILE AS A COMPONENT OF THE BABESIOSIS PATHOGENESIS

DOI: 10.36740/WLek202104119

Inna I. Torianyk
STATE INSTITUTION «MECHNIKOV INSTITUTE OF MICROBIOLOGY AND IMMUNOLOGY OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE», KHARKIV, UKRAINE

ABSTRACT

The aim is to identify the ultramicroscopic features of the erythrocytes as a component of the babesiosis pathogenesis using scanning electronic microscopy.

Materials and methods: Blood samples from 18 domestic dogs with clinically, microscopically, molecularly and genetically confirmed babesiosis served as the study material. The group of comparative control consisted of clinically healthy people (n=31) and domestic dogs (n=6). The method of scanning electron microscopy was used to study the spatial (three-dimensional coordinate system: the plane formed by the axes OX; OY; OZ) pathology of the size and shape of erythrocytes, microrelief of blood cells surfaces, membranopathies (raptures, macromolecules, conglomerates, micropores). The native peripheral blood smears of various origins were performed in accordance with the generally accepted method without fixation, staining/contrast. For this purpose, 0.01 ml of native blood was applied to the degreased surface of the metal platform (S=1 cm^2), dried in the air of the laboratory premise, analyzed (× 2000), using the resources of the equipment (SEO-SEM Inspect S50-B; energy dispersive spectrometer AZtecOne with detector X-MaxN20 (Oxford Instruments plc, UC) and power source in the gun: tungsten cathode with thermoelectron emission. This scanning electron microscope made it possible to examine the object under conditions of accelerating voltage of 100 kV. The analysis of the obtained results was performed in comparison.

Results: Under conditions of babesiosis development, the erythrocyte link of hematopoiesis responded by the reduction of the number of normocytes (99.0 % in clinically healthy people and domestic dogs, 77.3 % in dogs with babesiosis), increased of the number of regenerative forms of erythrocytes (1% in clinically healthy people and domestic dogs, 1,5 % in dogs with babesiosis), appearanced of degenerative forms of erythrocytes with characteristic pathology of cell size and shape, membranopathies (damage of the integrity of the membrane with the formation of defects).

Conclusions: Changes of the ultramicroscopic erythrocytes profile is one of the leading component of the babesiosis pathogenesis. The use of scanning electronic microscopy helps to obtain ultramicroscopic data on the presence of extraerythrocytic forms of Babesia on the background of the impossibility of diagnosis of anisochromia with detailing of inclusions in erythrocytes.

KEY WORDS: scanning electronic microscopy, erythrocytes, babesiosis, pathogenesis

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INTRODUCTION

Human babesiosis is a rapidly emerging, zoonotic, infectious disease caused by intra-erythrocytic protozoan parasites of the genus Babesia, which developed a complex life-cycle involving ticks as definitive hosts and vertebrates as intermediate hosts [1, 2]. Babesiosis is an increasing problem worldwide owing to the expansion of tick habitats and the increased mobility of animals, which promote the spread of parasites into new geographical areas [3].

In humans the disease manifestations are broad, from asymptomatic through mild flu-like infection to severe malaria-like disease with a potentially fatal outcome, mainly in immunedeficient and/or elderly individuals [4].

Hematologic manifestations of the disease are common. They can range from mild anemia, to severe pancytopenia, disseminated intravascular coagulopathy, or even hemophagocytic lymphohistiocytosis. Babesia parasites can be visualized on blood smears using the Giemsa-Wright stain. They are intraerythrocytic ring forms that resemble plasmodium, the causative agent of malaria. Babesia has extracellular merozoites [5]. Thus, blood examination, especially erythrocytes, is an important point in the diagnosis of babesiosis.

THE AIM

The aim is to identify the ultramicroscopic features of the erythrocytes as a component of the babesiosis pathogenesis using scanning electronic microscopy.

MATERIALS AND METHODS

Blood samples from 18 domestic dogs with clinically, microscopically, molecularly and genetically confirmed babesiosis served as the study material. The group of comparative control consisted of clinically healthy people (n=31) and domestic dogs (n=6), who underwent the blood cytological examination (light microscopy) preceding the scanning electronic microscopy, followed by verification of the results by resources of molecular genetic research (polymerase chain reaction).

The method of scanning electron microscopy was used to study the spatial (three-dimensional coordinate system: the plane formed by the axes OX; OY; OZ) pathology of the size and shape of erythrocytes, microrelief of blood cells surfaces, membranopathies (raptures, macromolecules, conglomerates, micropores). The native peripheral blood smears of various origins were performed in accordance with the generally accepted method without fixation, staining/contrast. For this purpose, 0.01 ml of native blood was applied to the degreased surface of the metal platform (S=1 cm^2), dried in the air of the laboratory premise, analyzed (× 2000), using the resources of the equipment (SEO-SEM Inspect S50-B; energy dispersive spectrometer AZtecOne with detector X-MaxN20 (Oxford Instruments plc, UC) and power source in the gun: tungsten cathode with thermoelectron emission. This scanning electron microscope made it possible to examine the object under conditions of accelerating voltage of 100 kV. The analysis of the obtained results was performed in comparison.

RESULTS AND DISCUSSION

Ultramicroscopic analysis of the superficial cytoarchitectonics of native erythrocytes of peripheral blood in clinically healthy people and dogs demonstrated their mesochromicity, electronic homogeneity of the stroma and presence of a semipermeable integral membrane. The cells were evenly distributed frontally, closely, but not densely, in the form of a rather mesodisperse population (fig. 1). Some loci contained clusters of hypochromic normocytes with enhanced central pallor (cyclicity of functional aspects of iron deficiency in women). The central pallor of the predominant number of normocytes was pronounced, contrasting, occupied less than one third of the diameter and indicated about the native mesochromia, corresponding to the shape of the biconcave disk (normodiskocyte). The tendency to sludge phenomenon (the phenomenon of «coin columns») was minimized. The level of reticulocytes did not exceed 1 % (table 1).

The generalized results of scanning electron microscopy of peripheral blood of domestic dogs of rural and suburban areas of Kharkiv, Poltava and Sumy regions (Ukraine) with babesiosis showed that the population of erythrocytes formed disparate heterogeneous, extremely hyperchromic, expressively pronounced contrast clusters of cells, concentrated in the frontal plane of the preparations. The density of cells was low, with numerous intervals of spare areas (fig. 2). Intercellular contacts were preserved owing to membranes. General image format was determined by single clusters of cells. Among erythrocytes ovalo-, sphero-, macroovalocytes, fragmented cells prevailed. Dacryo-, echinocytes, bladdery cells were organized in separate loci. The dispersed spectrum was completed by cells with expressed heterogeneity of electron density of the stroma. In such cases, the central segments of erythrocytes contained obscurations of ovoid, spherical (from one third of erythrocytes), pear-shaped forms, which tended with blunt ends to one of the poles of the cell. Extraerythrocyte layers were extremely rare. The appearance of foreign formations of spatial configurations, representative of the causative agents of babesiosis, in the areas close to erythrocytes was local (occasional cases). Supramembrane implantation of pathogens was not detected. Indentations, membrane-stromal defects, pores were exceedingly rare. Pre-hemolytic, hemolytic cells were visualized. Cellular detritus was minimized, represented by polymorphic fragments of erythrocytes (schistocytes). Disposal of the latter took place in due time, but without obvious signs of erythrophagia. The cytological plot was completed with the appearance of single «erythrocyte rosettes/semi-rosettes» of 7-9 cells and a centered normocyte, chains of 6-8 blood corpuscles. The sludge phenomenon occurred due to poikilocytes (from 6 to 11 elements). Oligocythemia was caused by an expressed decrease in the number of peripheral blood erythrocytes. Thrombocyte plates in the fields of view of slides were extremely rare. The level of reticulocytes was 1.5 % (table 1).

Summarizing the above, it can be noted that in babesiosis an increase the already high structural and functional vulnerability of blood erythrocytes was objectively explained by the lack of repair systems and low adaptive potential. The cells were not able to synthesize proteins and lipids de novo, participate in oxidative phosphorylation, maintain the cycle of tricarboxylic acids. Energy was obtained anaerobically according to Embden-Meyerhof pathway, traditionally accumulating the latter in the form of adenosine triphosphate [6]. One of the ways to carry out the functional activity of erythrocytes in response to oxidative stimulation was focused on the resources of hexose monophosphate shunt [7]. Following this, the life cycle of the damaged cells continued. However, the influence of exogenous factors (infectious) did not stop and inevitably led to deep (decompensated) changes in the surface architecture, intracellular balance. Anomalies of membrane proteins of the cytoskeleton of erythrocytes (spectrin, ankyrin, segment 3, protein 4.1) led to the changes in the spatial configuration / surface cytoarchitectonics of normal discocytes with the transformation of the latter into regenerative, mostly into degenerative forms (sphero-, ovalo-, schisto-, stomatocytes, etc.) [2].

Another consequence of abnormalities in the cytoskeleton of erythrocytes was the corresponding clinical profile, characterized by hemolytic anemia and splenomegaly, spherocytosis in peripheral blood. The appearance of spherocytes occurred during the presence of erythrocytes with abnormal structural proteins of the membrane in the spleen, when the cells lost not only fragments of the membrane (ruptures), but also the normal biconcave shape (erythrocytes took the form of a ball). Presence of ovalocytes (elliptocytes) was caused by cytoskeletal abnormalities in the areas of interaction between cytoplasmic proteins (α- and β-spectrins, protein 4.1). Clinical and morphological support was characterized by moderate anemia, splenomegaly, microspherocytosis, and presence of schistocytes (fragmented forms of erythrocytes). Detection of acanthocytes (spur-like cells with membrane protrusion) was based on the initiation of the synthesis of abnormal

Table 1. The relative indicators (%) of morphological forms of erythrocytes in blood.

Group name	Normocytes (discocytes)	Reticulocytes	Pathology of shape and size of erythrocytes
Clinically healthy individuals and domestic dogs	99.0 %	1.0 %	0 %
Domestic dogs with detected babesiosis	77.3 %	1.5 %	21.2 %

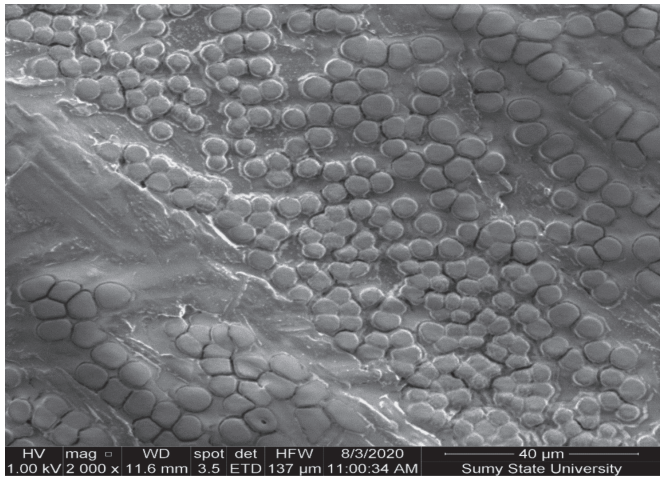

Fig. 1. Erythrocytes of native blood of clinically healthy person in the frontal plane. Scanning electron microscopy, × 2000.

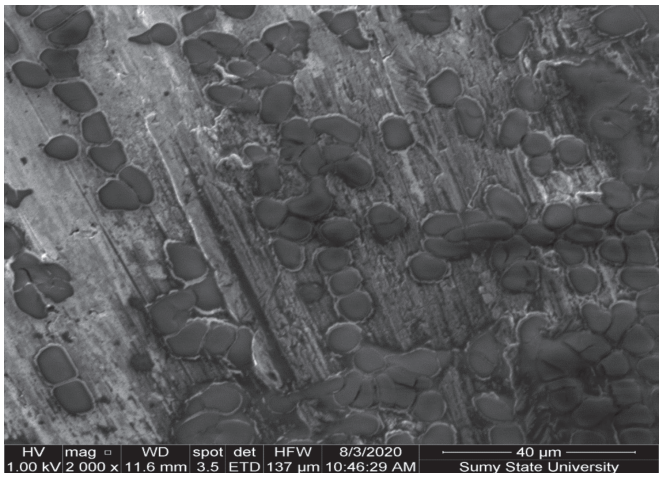

Fig. 2. Erythrocytes of native peripheral blood of a domestic male dog with detected babesiosis. Macroovalocytes, echinocytes, fragmented cells, bladdery cell, erythrocytes with blackening in the cytoplasm. Scanning electron microscopy, × 2000.

plasma lipoproteins and erythrocyte membrane. Registration of these types of erythrocytes indicated profound functional disorders of the liver that occurred in response to massive parasitic aggression. The presence of hematuria and uremia provoked the release of echinocytes into the blood (hedgehog-like, spear-like forms of erythrocytes). Sometimes the appearance of these cells in laboratory tests preceded the expressed clinical symptoms of babesiosis, regarded as one of the early nonspecific diagnostic criteria, a prodrome of upcoming uremia [8, 9].

Postinvasive aniso- and poikilocytosis of erythrocytes significantly affected the rheological properties of blood (as shown by morphological observations, they, among other things, had a significant contribution to the start of the reactions of intravascular blood coagulation). Aggressive invasion of pathogens of blood-parasitic babesiosis to the organism of mammals provoked the program of «emergency» start of intense erythropoiesis. An objective confirmation of the latter was the increase in the content of young reticulocytes. This indicator increased due to the absolute rise in the number of reticulocytes or as a consequence of a decrease in the mass of circulating erythrocytes (anemia) [10-12].

The next component in the pathogenetic picture of babesiosis development was the migration into the bloodstream of numerous erythrocytes with altered adhesive properties of the cell membrane, in addition, protein complexes adsorbed on it. Hyperaggregation of erythrocytes in vivo ad oculi led to post-invasive formation of erythrocyte sludges, aggregates-microthrombi in the blood. Appearance of the latter in the microcirculatory bed inevitably caused complete or partial blockage of blood circulation in certain areas of the tissues of target organs, sharply reduced the number of actively functioning capillaries, arteriolo-venular anastomoses, contributed to the development of tissue hypoxia, atrophy, necrosis [12-14].

CONCLUSIONS

Changes of the ultramicroscopic erythrocytes profile is one of the leading component of the babesiosis pathogenesis. Under conditions of babesiosis development the erythrocyte link of hematopoiesis responds by reduction of the number of normocytes, an increase of the number of regenerative forms of erythrocytes, appearance of degenerative forms of erythrocytes with characteristic pathology of cell size and shape, membranopathies (damage of the integrity of the membrane with the formation of defects). The use of scanning electronic microscopy helps to obtain ultramicroscopic data on the presence of extraerythrocytic forms of Babesia on the background of the impossibility of diagnosis of anisochromia with detailing of inclusions in erythrocytes.

REFERENCES

1. Suarez CE, Alzan HF, Silva MG, Rathinasamy V, Poole WA, Cooke BM. Unravelling the cellular and molecular pathogenesis of bovine babesiosis: is the sky the limit? International Journal of Parasitology. 2019;49(2):183-197.
2. Rossouw I, Maritz-Olivier C, Niemand J, van Biljon R, Smit A, Olivier NA, Birkholtz L-M. Morphological and molecular descriptors of the developmental cycle of Babesia divergens parasites in human erythrocytes. PloS Neglected Tropical Diseases. 2015;9(5): e0003711. doi:10.1371/journal.pntd.0003711.

3. Beugnet F, Moreau Y. Babesiosis. Revue scientifique et technique (International Office of Epizootics). 2015;34(2):627-639.
4. Ord RL, Lobo CA. Human babesiosis: pathogens, prevalence, diagnosis and treatment. Current Clinical Microbiology Reports. 2015;2(4):173-181.
5. Akel T, Mobarakai N. Hematological manifestations of babesiosis. Annals of Clinical and Laboratory Research. 2017;5(1). doi: 10.21767/2386-5180.1000149.
6. Ronimus RS, Morgan HW. Distribution and phylogenies of enzymes of the Embden-Meyerhof-Parnas pathway from archaea and hyperthermophilic bacteria support a gluconeogenic origin of metabolism. Archaea. 2003;1:199-221.
7. Akram M, Shah SMA, Munir N, Daniyal M, Tahir IM, Mahmood Z, Irshad M, Akhlaq M, Sultana S. Hexose monophosphate shunt, the role of its metabolites and associated disorders: a review. Journal of Cellular Physiology. 2019;234(9):14473-14482.
8. Magni R, Luchini A, Liotta L, Molestina RE. Proteomic analysis reveals pathogen-derived biomarkers of acute babesiosis in erythrocytes, plasma, and urine of infected hamsters. Parasitology Research. 2020;119:2227-2235.
9. Sevilla E, González LM, Daniel L, Gray J, Montero E. Kinetics of the invasion and egress processes of Babesia divergens, observed by time-lapse video microscopy. Scientific Reports. 2018;8:14116. doi: 10.1038/s41598-018-32349-7.
10. Piane L, Theron M-L, Aumann M, Trumel C. Spurious reticulocyte profiles in a dog with babesiosis. Veterinary Clinical Pathology. 2016;45(4):594-597.
11. Parodi E, Giraudo MT, Ricceri F, Aurucci ML, Mazzone R, Ramenghi U. Absolute reticulocyte count and reticulocyte hemoglobin content as predictors of early response to exclusive oral iron in children with iron deficiency anemia. Anemia. 2016;7345835. https://doi.org/10.1155/2016/7345835.
12. Zelya OP, Kukina IV. Babezioz cheloveka. Human babesiosis. Medical News of North Caucasus. 2020;15(3):449-455. (Ru).
13. Ortiz JF, Millhouse PW, Cox ÁM, Campoverde L, Kaur A, Wirth M, Atoot A. Babesiosis: appreciating the pathophysiology and diverse sequela of the infection. Cureus. 2020; 12(10): e11085. doi: 10.7759/cureus.11085.
14. Rafaj RB, Kules J, Selanec J, Vrkić N, Zovko V, Zupančič M, Bakija AT, Matijatko V, Crnogaj M, Mrljak V. Markers of coagulation activation, endothelial stimulation, and inflammation in dogs with babesiosis. Journal of Veterinary Internal Medicine. 2013;27:1172-1178.

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ORCID and contributionship:
Inna I. Torianyk: 0000-0001-6843-8808 [A,B,C,D,E,F]

Conflict of interest:
The Author declare no conflict of interest.

CORRESPONDING AUTHOR
Inna I. Torianyk
Laboratory of Viral Infections,
State Institution «Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine»
39 Kamysheva Ivana str., apt. 9, 61038, Kharkiv, Ukraine
e-mail: pathologynew@ukr.net

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ORIGINAL ARTICLE

A STATISTICAL ANALYSIS OF OBSTETRIC AND PERINATAL COMPLICATIONS IN SINGLETON AND MULTIPLE PREGNANCIES ONCE ASSISTED REPRODUCTIVE TECHNOLOGIES ARE USED

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Tamara H. Romanenko[1], Olha M. Sulimenko[1,2], Svitlana O. Ovcharenko[2]
[1]SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION THE MINISTRY OF HEALTH OF UKRAINE, KYIV, UKRAINE
[2] LELEKA MATERNITY HOSPITAL LLC, KYIV, UKRAINE

ABSTRACT

The aim: To carry out a comparative statistical analysis of obstetric and perinatal complications in singleton and multiple pregnancies once assisted reproductive technologies (ART) are applied according to the records taken from archival materials (maternity and delivery records) and identify the clinical features of multiple pregnancy.

Materials and methods: Over the period of 2017-2019, 522 women gave birth in LELEKA Maternity Hospital LLC after using assisted reproductive technologies and 331 women among them were followed-up in the women's health center of LELEKA maternity hospital. Among these women (522) with singleton pregnancy 445 women gave birth, while in multiple pregnancy – 77.

The statistical analysis of 150 maternity and delivery records was carried out. All pregnant women were divided into two groups: group 1 – 75 women having singleton pregnancy after ART; group 2 – 75 women having multiple pregnancy after ART. Women getting pregnant after ART, or in vitro fertilization (IVF) and five-day frozen embryo transfer to be exact, turned out to be the selection criterion for a comparative statistical analysis. Mathematical methods for research were used as O.P. Mintser (2013) suggested. The reliability of the digit cancellation test was calculated using the Fisher's exact test and Student's T-test. Graphs were designed using Microsoft Excel.

Results: The complications of early multiple pregnancy were the following: anemia (47.8% as opposed to 22.9%, $p<0.01$), placental insufficiency (43.3% in contrast to 22.9%, $p<0.01$), threatened abortion (41.8% in contrast to 28.6%, $p<0.01$). The complications in late pregnancy are as follows: preeclampsia (52.7% as opposed to 20.6%, $p<0.01$), intrauterine growth restriction (20.0% as opposed to 7.4%, $p<0.01$), anemia in pregnancy (76.4% in contrast to 32.4%, $p<0.01$), placental insufficiency (47.3% in contrast to 22.1%, $p<0.05$).

Conclusions: Multiple pregnancy is a high risk for anemia in pregnancy, preeclampsia, placental insufficiency, early intrauterine growth restriction and fetal distress in pregnancy and labor. It predetermines the high level of a caesarean section. Therefore, further research aimed at prediction and prevention of obstetric and perinatal complications in multiple pregnancy after ART is currently topical.

KEY WORDS: obstetric and perinatal complications, multiple pregnancy, assisted reproductive technologies

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INTRODUCTION

The use of assisted reproductive technologies (ART) enables to effectively fight infertility off over the past 40 years. More than 5 million infants worldwide were born once ART had been applied in infertility [1, 2]. However, getting pregnancy using ART is only the first stage, while the birth of a healthy newborn depends on the course and management of pregnancy and childbirth for sure. Characteristics of multiple pregnancy, once ART is applied, hold an exceptional interest for applied science, at the stages of embryo implantation and placentation in particular. Thus, despite the widespread adoption of ART for assisted conception, the matter of enhancing the ART effectiveness still remains relevant, as well as optimizing the management of labor and delivery since the early gestational age in order to minimize risks for both a mother and her child. Just over the last couple of years the incidence of multiple pregnancy rapidly grew, and ranges from 0.7% to 1.5% in the majority of European countries. According to the registries by the European Society of Human Reproduction and Embryology (ESHRE) the effectiveness of ART programs today is nearly 40%. More than 1.8 million children were born in Ukraine in 2019, 27 thousand (1.6%) of which – after ART program [1, 2]. The occurrence of twin pregnancy in the natural cycle is low and according to different researchers, ranges from 11 to 14 per 1000 deliveries, the use of ART led to a 20-time increase in the number of multiple pregnancies comparing to a natural population [2]. Over the last 10 years, the number of deliveries of twins increased by 27%, triplets – by 38%. These days iatrogenic multiple pregnancy comprises 30-80% among multiple pregnancies. Delivery of twins account for 20-30%, triplets – 4-6% among pregnancies that develop after ART. It is no secret that multiple pregnancy is associated with a high rate of adverse perinatal outcomes [2-5]. Statistics show that only 11-15% of multiple pregnancies and labors occur without complications. Perinatal mortality in delivery of more than one is 3 to 7 times higher than in singleton pregnancy [2-

6]. It is known that multiple pregnancy itself determines a high risk of perinatal pathology. The high prevalence of threatened abortion with retroplacental hematoma as comorbidity is observed (the incidence of retrochorial hematoma during the first trimester in pregnancies after ART comprised 13.5%) [7]. The prevalence of miscarriage in pregnancy after ART is 18-44.4%, while the prevalence of preterm labor ranges from 19.5% to 37.6%, the prevalence of gestational hypertension after ART was registered in 5.9% in singleton pregnancy and in 12.6% in multiple pregnancy. According to ACOG [2,6-11] the higher risk of preeclampsia (OR 2.7) turned out to be associated with ART [2, 6-11]. Preeclampsia (PE) still remains a global health issue, threatens maternal health and life [2, 11]. PE holds 9-26% of maternal mortality and high percentage of preterm labor, predetermines both maternal and neonatal morbidity [2, 12]. Statistics suggest that the prevalence of severe PE in multiple pregnancy is 3-4 times higher than in singleton pregnancy [2, 11, 13-16].

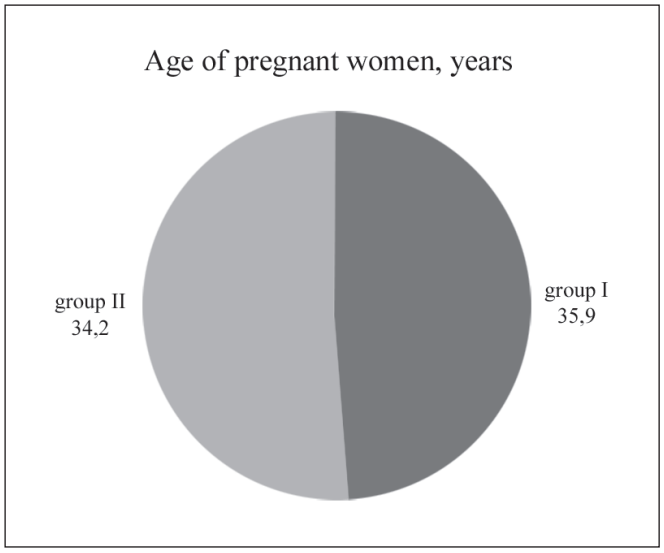

Fig. 1. Mean age of women examined (year M±m)

THE AIM

To carry out a comparative statistical analysis of obstetric and perinatal complications in singleton and multiple pregnancies once assisted reproductive technologies (ART) are used according to the records taken from archival materials (maternity and delivery records) and identify the clinical features of multiple pregnancy.

MATERIALS AND METHODS

Over the period of 2017-2019, 522 women gave birth in LELEKA Maternity Hospital LLC after using assisted reproductive technologies and 331 women among them were followed-up in the women's health center of LELEKA maternity hospital. Among these women (522) with singleton pregnancy 445 women gave birth, while in multiple pregnancy – 77.

The statistical analysis of 150 maternity and delivery records was carried out. All pregnant women were divided into two groups: group 1 – 75 women having singleton pregnancy after ART; group 2 – 75 women having multiple pregnancy after ART. Women getting pregnant after ART, or in vitro fertilization (IVF) and five-day frozen embryo transfer to be exact, turned out to be the selection criterion for a comparative statistical analysis. Mathematical methods for research were used as O.P. Mintser (2013) suggested. The reliability of the digit cancellation test was calculated using the Fisher's exact test and Student's T-test. Graphs were designed using Microsoft Excel.

RESULTS AND DISCUSSION

The majority of women examined were women aged 25-45 with infertility lasting 3-9 years. The mean age of women did

Table I. Extragenital diseases in women examined (abs. n., %)

Extragenital diseases	Groups of women	
	Group 1 n=75	Group 2 n=75
Diabetes mellitus	2 (2.7%)	5 (6.7%)*
Rheumatoid arthritis, congenital heart defects	2 (2.7%)	2 (2.7%)
Hypertension	2 (2.7%)	5 (6.7%)*
Chronic obstructive pulmonary diseases	2 (2.7%)	3 (4%)
Chronic urinary tract infections	6 (8%)	7 (9.3%)
Hepatitis and cholecystitis	6 (8%)	7 (9,3%)
Varicose veins	5 (6.7%)	4 (5.3%)
Anemia	6 (8%)	10 (13.7%)*
Myopia	1 (1.3%)	2 (2.7%)
Thyroid disease	7 (9.3%)	11 (14.7%)*
Overall	39 (52%)	56 (74.7%)

Statistical significance of differences comparing to group 1; *$p<0.05$

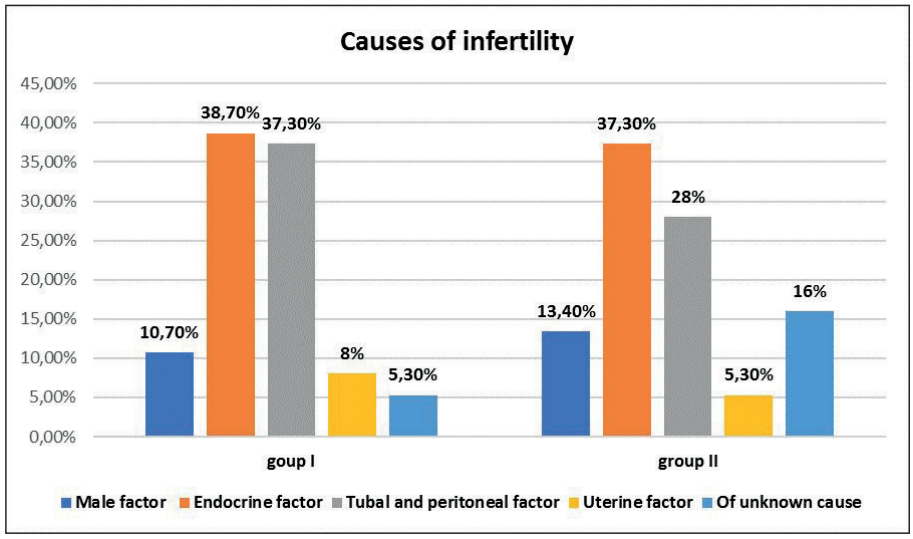

Fig. 2. Infertility causes in women examined (%)

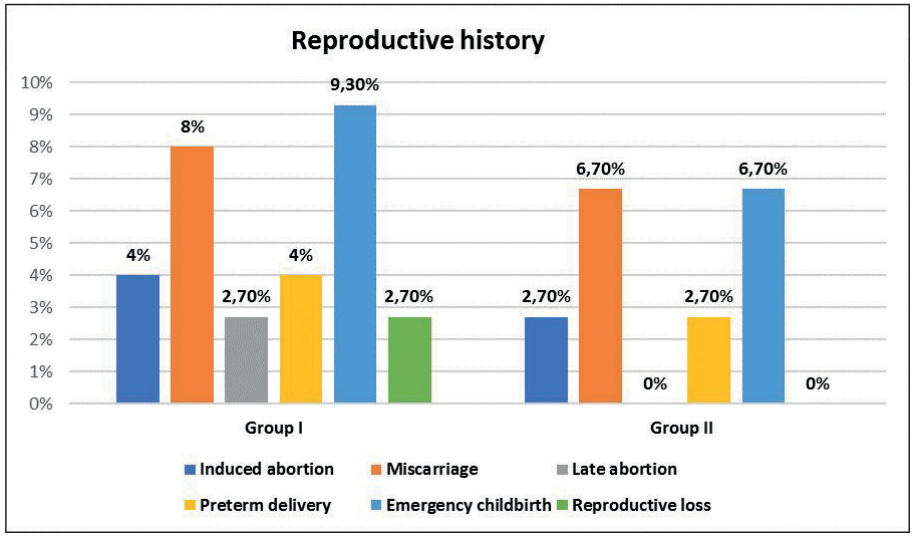

Fig. 3. Reproductive history (%)

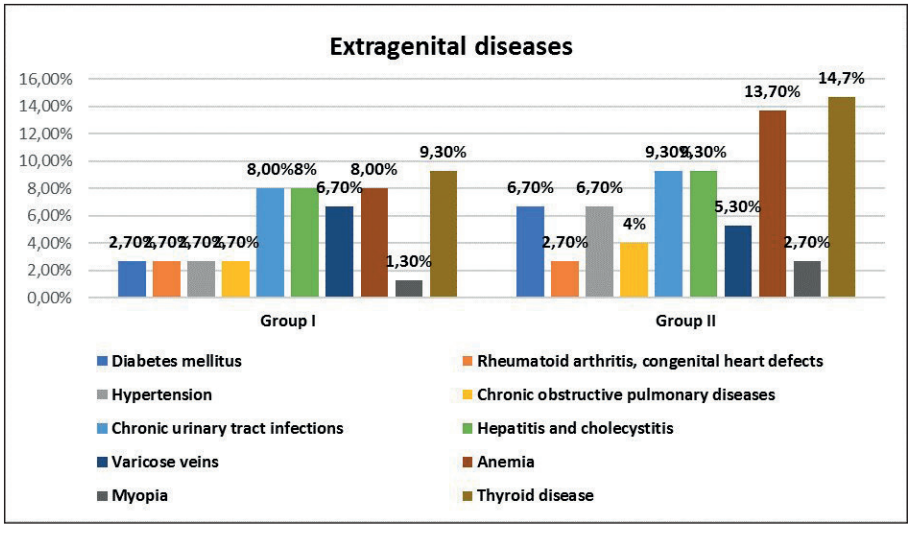

Fig. 4. Extragenital diseases (%)

not differ significantly by groups and turned out to be statistically insignificant (p>0.05) as study results attest (Fig. 1).

Thus, the age of women having singleton pregnancy (group 1) averaged out at 34.2 ± 2.1 years. The mean age of women in group 2 was 35.9 ± 2.2 years. All women having induced pregnancy in groups 1 and 2 were registered in the women's health center.

Primary infertility was observed in 67.3% of all women examined, secondary infertility – in 32.7%, the mean age of infertility was 3-6 years. It is worth mentioning that primary

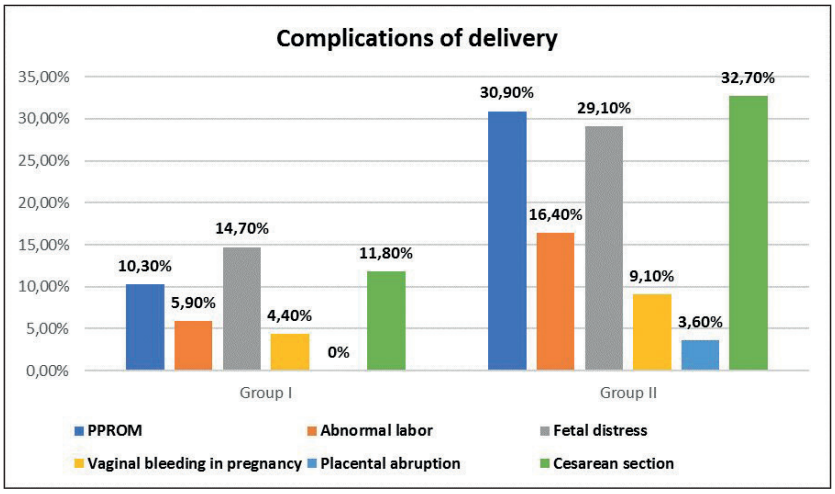

Fig. 5. Complications of delivery (%)

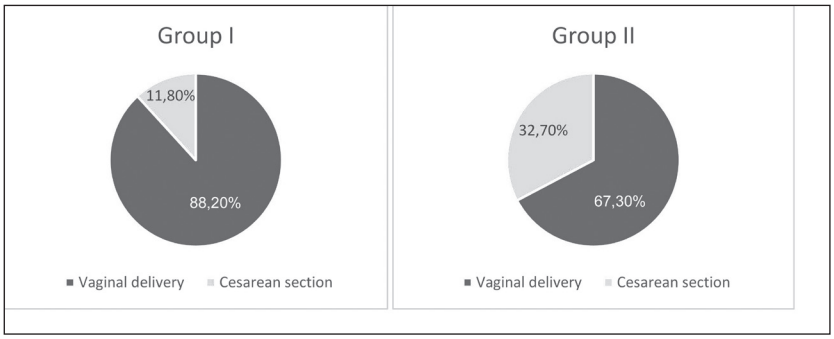

Fig. 6. Principles of delivery in women examined (%)

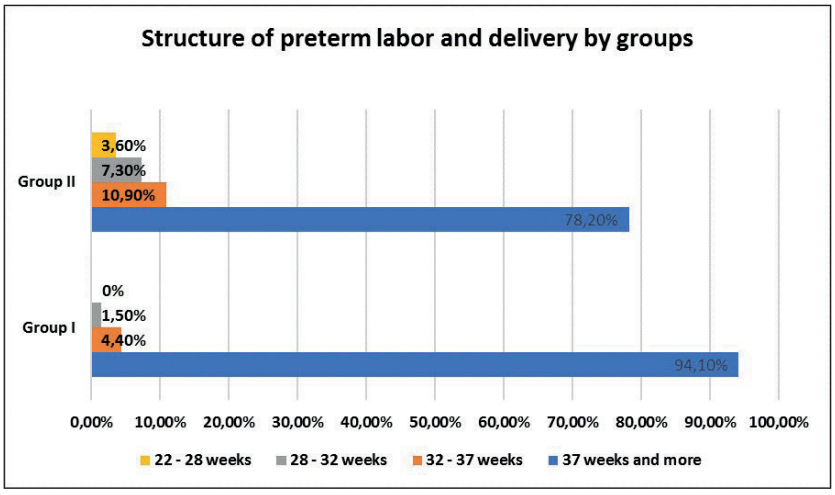

Fig. 7. Structure of preterm labor by groups (%)

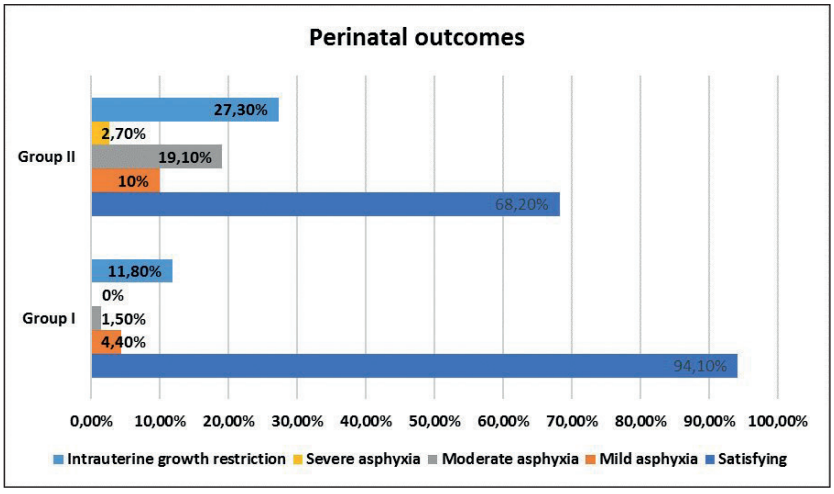

Fig. 8. Perinatal outcomes of birth (%)

Table II. Clinical features of early pregnancy (abs. n., %)

Complications	Groups of women	
	Group I n=70	Group II n=67
Vomiting in pregnancy	8 (11.4%)	11 (16.4%)
Threatened abortion	20 (28.6%)	28 (41.8%)**
Anemia in pregnancy	16 (22.9%)	32 (47.8%)**
Placental insufficiency	16 (22.9%)	29 (43.3%)**
Exacerbation of a physical disorder	8 (11.4%)	11 (16.4%)

Statistical significance of differences comparing to group 1; *p<0.05; **p<0.01

Table III. Clinical features of late pregnancy (abs. n., %)

Complications	Groups of women	
	Group I n=68	Group II n=55
Threatened abortion	16 (23.5%)	31 (56.4%)*
Anemia in pregnancy	22 (32.4%)	42 (76.4%)**
Preeclampsia	14 (20.6%)	29 (52.7%)**
Gestational hypertension	6 (8.8%)	10 (18.2%)*
Gestational diabetes	10 (14.7%)	13 (23.7%)*
Placental insufficiency	15 (22.1%)	26 (47.3%)*
Polyhydramnios	4 (5.9%)	15 (27.3%)
Cervical weakness	2 (2.9%)	7 (12.7%)*
Exacerbation of a physical disorder	9 (13.2%)	9 (16.4%)
IUGR	5 (7.4%)	22 (20%)** n=110
Low-lying placenta	1 (1.5%)	1 (1.8%)

Statistical significance of differences comparing to group 1; *p<0.05; **p<0.01

Table IV. Structure of indications for Cesarean section (abs. n. %)

Indications	Group I n = 8	Group II n = 18
IUGR	1 (12.5%)	2 (11.1%)
Fetal distress	3 (37.5%)	2 (11.1%)
Preeclampsia	-	5 (27.8%)
Abnormal labor	3 (37.5%)	-
Placental abruption	-	3 (16.7%)
Others	1 (12.5%)	6 (33.3%)

Statistical significance of differences comparing to group 1; *p<0.05

infertility prevailed in women from group 2, in contrast to group 1 where secondary infertility prevailed. Infertility causes didn't vary by groups (p<0.05) and were categorized as follows (Fig. 2).

The findings of fertility health check are illustrated in figure 3, they indicate the absence of significant difference in reproductive history. At the same time, both groups are reported to have a low level of induced abortions (4% as opposed to 2.7%) and early threatened abortions (8% in contrast to 6.7%) (p>0.05).

The level of reproductive loss in the past history (group 2 – absent, group 1 – 2.7%) and preterm labor (2.7% in group 2 and 4.0% in group 1) are indicative. It can be explained by a significantly higher incidence of primary infertility in group 2 comparing to group 1 (p<0.05).

Regarding extragenital disease patterns in women examined we traced a significantly higher prevalence of diabetes mellitus, hypertension, anemia, thyroid diseases (p<0.05) in pregnant women of group 2 as opposed to group 1 (Table I).

According to our findings, the prevalence of extragenital diseases in women of group 1 comprised 74.7% – 56 women in contrast to 52% – 39 women of group 2. This

group included women with cardiovascular, pulmonary, endocrine, urologic and gastrointestinal diseases. Based on the prevalence and pattern of extragenital diseases the following diseases were of a significantly higher occurrence in group 2: diabetes mellitus, hypertension, anemia and thyroid diseases that, in our opinion, increase the risk of preeclampsia in women having multiple pregnancy.

The occurrence of extragenital diseases as comorbidity in groups studied was illustrated in figure 4.

Thus, es evidenced by the study of premorbidity in groups of women having singleton and multiple pregnancies after ART we recognized the factors in women in case of multiple pregnancy that predispose to obstetric and perinatal complications in pregnancy. Data on gestation clinical features are very indicative when comparing (Table II). The main complications of early pregnancy in case of multiple pregnancy were: anemia (47.8% as opposed to 22.9%, p <0.01), placental insufficiency (43.3% in contrast to 22.9%, p <0.01), threatened abortion (41.8% in contrast to 28.6%, p <0,01). It is worth mentioning that in 36% of pregnant women in group 1 and 42.7% in group 2 (p<0.05) retroplacental hematomas are reported. Pregnancy ended up as miscarriage in 3 (4%) pregnant women of group 1 during the first trimester and missed miscarriage in 2 (2.7%) cases; 4 (5.3%) cases of miscarriage and 4 (5.3%) – missed miscarriage of the first trimester (p<0.05).

The same pattern took place after 20 weeks of pregnancy (Table III). Thus, 2 (2.9%) cases of late abortion took place in group 1 as opposed to 7 (10.5%) cases in group 2, (p<0.05). Three (5%) cases of intrauterine death of both fetuses in patients of group 2 at 22-24 weeks should also be noted, as well as intrauterine death of only one fetus in 2 (3.3%) cases, one case at 23 weeks and the other one at 28.

Various complications of pregnancy were observed almost in all women having multiple pregnancy. Thus, anemia in pregnancy was the most common – 42 (76.4%) cases, threatened abortion – 31 (56.4%) cases, preeclampsia – 29 (52.7%) cases, placental insufficiency – 26 (47.3%) cases, which led in the majority of cases to intrauterine growth restriction in 22 infants (20%)**.

In comparison, the occurrence of these complications in group 1, in singleton pregnancy, was substantially lower: anemia in pregnancy- 22 (32.4%) cases, threatened abortion – 16 (23.5%) cases, preeclampsia of various severity – 14 (20.6%) cases and placental insufficiency – 15 (22.1%), intrauterine growth restriction – 5 (7.4%). The number of preeclampsia cases in women having multiple pregnancy was 2.6 times higher than in singleton pregnancy and comprised 52.7% (29) in contrast to 20.6% (14) in group 1 (p<0.01).

In multiple pregnancy placental insufficiency manifested as IUGR in 5 (19.2%) patients, dissociated fetal development in 4 (15.4%), while in 6 (23.1%) cases dissociated fetal development was combined with growth restriction. Chronic placental insufficiency was compensated and confirmed by the findings of morphological analysis in 11 (42.3%) cases.

As illustrated by figure 5, five main complications of delivery in women having multiple pregnancy were: premature rupture of membranes (30.9% as opposed to 10.3%, p<0.05), abnormal labor (16.4% as opposed to 5.9%, p>0.05), fetal distress (29.1% in contrast to 14.7%, p<0.05), placental abruption (3.6% as opposed to it's absence in group 1). This led in turn to an increase in the rate of Cesarean sections in women in case of multiple pregnancy.

Based on the data obtained when assessing delivery techniques, a significant increase in the rate (32.7%) of Cesarean section in case of multiple pregnancy in contrast to 11.8% of patients in singleton pregnancy (p<0.01) should be noted (Fig.6).

Interestingly, the structure of indications for Cesarean section in women having multiple pregnancy (Table IV) was as follows: preeclampsia progression (27.8%), IUGR (11.1%), fetal distress (11.1%), (16.7%) – placental abruption, other indications (breech and trunk presentation, oblique lie, clinical narrow pelvis, uterine scar) – 33.3%. the Principal indications for Cesarean section in women of group 1 were the following: fetal distress with placental insufficiency as comorbidity (37.5%), ineffective treatment of abnormal labor (37.3%), other indications (breech and trunk presentation, oblique lie, clinical narrow pelvis, uterine scar) – 12.5%.

The analysis of birth outcomes in all groups revealed that in patients of group 2 multiple pregnancy 3.7 times as often ended up prematurely as singleton (21.8% and 5.9%, p <0.05). Almost half of the cases were deliveries at 34 weeks according to a gestation period, in other words, early preterm labor prevailed, 3.6% of which were deliveries at 22-28 weeks, 7.3% – at 28-32 weeks and 6.4% – at 32-34 weeks (Fig. 7).

The average gestation period in singleton pregnancy herein comprised 37.6 ± 1.2 weeks, 35.1 ± 1.3 weeks – in multiple. Data on perinatal outcomes of birth are of substantial interest. When assessing perinatal outcomes of birth (Figure 8) a relevant increase in the sum rate of birth asphyxia of various severity in case of multiple pregnancy (35.0% in contrast to 5.9%, p <0.05), intrauterine growth restriction (27.3% vs. 11.8%, p <0.01) should be noted. Further, it affected the rate of early neonatal morbidity with delayed post-hypoxic leukoencephalopathy (23.6%) being predominant in group 2; intrauterine infection (12.7%); hemorrhagic syndrome (9.1%) and hyperbilirubinemia (12.7%).

Thus, according to the findings obtained in statistical analysis of delivery records in women having multiple pregnancy, 2 cases of miscarriage/stillbirth (2nd fetus in both cases) took place. Congenital brain malformation, anencephaly to be exact, was a primary disease in the first case. Fetal death occurred in utero. The birth of a second child happened at 38 week of pregnancy, the live girl 2890 g of weight and 51 cm of height was born, Apgar score of 6-7. In the other case, the cause of miscarriage/stillbirth was perinatal asphyxia (antepartum cause) in women with preeclampsia and severe placental insufficiency followed by intrauterine growth restriction. The pregnant woman gave birth at 34 weeks due to arising signs of severe preeclampsia, the live boy

2050 g in weight, 48 cm in height was born prematurely, Apgar score of 5-6.

Two newborns died in early neonatal period (newborn twins). The mother delivered 3 prematurely at 32-33 weeks, placental abruption, moderate preeclampsia, severe anemia. Cesarean section was performed, two live girls were born 1600 g and 1840 g in weight, 39 cm and 41 cm in height, Apgar score of 3-3 and 2-2, respectively, stayed alive for 2 hours 5 minutes and 1 hour 17 minutes. Granulomatous Amebic Encephalitis as an intrauterine infection with chorioamnionitis was the primary disease that led to placental disruption and severe asphyxia in children.

In our opinion such a high occurrence of obstetric complications in women having multiple pregnancy results from a high risk in multiple pregnancy, and the course of pregnancy and labor in such patients is followed by a great number of complications that are way more severe than in singleton pregnancy. Literature indicates that multiple pregnancy develops in the presence of a greater number of complications than singleton. The most common and typical in multiple pregnancy are the following complications: threatened abortion, anemia, preeclampsia, placental insufficiency, intrauterine growth restriction, miscarriage & stillbirth of one or both fetuses [1, 2, 11]. The above-mentioned clinical features of multiple pregnancy for sure directly affected obstetric and perinatal outcomes of birth in such women.

CONCLUSIONS

The findings of the statistical analysis of archival delivery records enable to conclude about:

1. Multiple pregnancy is a high risk for anemia in pregnancy (76.4%), preeclampsia (52.7%), placental insufficiency (47.3%), early intrauterine growth restriction (27.3%) and fetal distress in pregnancy and labor (29.1%).
2. The high rate of obstetric and perinatal complications in these women leads to the high rate of Cesarean section (32.7%) and asphyxia cases in newborns (35%).
3. Due to the low effectiveness of widely accepted methods for prediction, treatment and prevention of obstetric and perinatal complications in multiple pregnancy after ART, further research is demanded.

REFERENCES

1. Kamphuis E.I., Bhattacharya S., van der Veen F. at al.; Evidence Based IVF Group. Are we overusing IVF? BMJ. 2014; 348: 252.
2. American College of Obstetricians and Gynecologists. "Perinatal risks associated with assisted reproductive technology: Committee Opinion No. 67." J Obstet Gynecol. 2016;128: e61-8.
3. Di Nisio M., Ponzano A., Tiboni G.M. et al. Effects of multiple inherited and acquired thrombophilia on outcomes of in-vitro fertilization. Thromb Res. 2018; 9 (167):26-31. DOI: 10.1016/j.thromres.2018.05.006.
4. Chighizola C.B., Raimondo M.G., Meroni P.L. Does APS Impact Women's Fertility? Curr Rheumatol Rep. 2017;(6):33. DOI: 10.1007/s11926-017-0663-7.
5. Vermey B.G., Buchanan A., Chambers G.M. et al. Are singleton pregnancies after assisted reproduction technology (ART) associated with a higher risk of placental anomalies compared with non-ART singleton pregnancies? A systematic review and meta-analysis. BJOG. 2018. doi: 10.1111/1471-0528.15227.
6. Barinov S.V., Rogova E.V., Dolgich T.I. et al. Osobennosti techeniya mnogoplodnoj beremennosti v sochetanii s trombofiliyami [Features of o course of polycarpous pregnancy in combination with trombophylia]. Mother and child in Kuzbass. 2014: 2(57): 39-42. (in Russian).
7. Xiang L., Wei Z., Wu J. at al. Clinical significance of first-trimester intrauterine haematomas detected in pregnancies achieved by IVF-embryo transfer. Reprod Biomed Online. 2014;4:445-51. doi: 10.1016/j.rbmo.2014.06.015.
8. Adamyan L.V., Arty`muk N.V., Bashmakova N.V. et al Gipertenzivny`e rasstrojstva vo vremya beremennosti, v rodakh i poslerodovom periode. Pree`klampsiya. E`klampsiya. Klinicheskie rekomendaczii. [Hypertensive disorders during pregnancy, childbirth and the postpartum period. Preeclampsia. Eclampsia. Clinical guidelines]. Treatment protocol. 2016: 40. (in Russian).
9. Ul`yanina E.V., Fatkullin I.F., Khajrullina G.R. Markery` angiogeneza i ul`trazvukovogo issledovaniya v oczenke stepeni tyazhesti sindroma zaderzhki razvitiya ploda. [Markers of angiogenesis and ultrasound in assessing the severity of fetal growth retardation syndrome]. The Bulletin of Contemporary Clinical Medicine. 2016;9(5): 79-82. (in Russian).
10. Townsend R., O'Brien P., Khalil A. Current best practice in the management of hypertensive disorders in pregnancy. Integr. Blood Press Control. 2016;9:79-94. doi: 10.2147/IBPC. S77344.
11. Barinov S.V., Rogova E. V., Kadczy`na T. V. et al. Prognozirovanie riska tyazheloj pree`klampsii pri mnogoplodnoj beremennosti i puti ee profilaktiki. [Prediction of the risk of severe pre-eclampsia in multiple pregnancies and ways of its prevention.] .Bulletin of the Peoples' Friendship University of Russia. Series: Medicine. 2015;1:69–75. (in Russian).
12. Okun N., Sierra S. et al. Pregnancy outcomes after assisted human reproduction. Society of Obstetricians and Gynaecologists of Canada J. Obstet. Gynaecol. Can. 2014;36 (1):64-83.
13. El-Toukhy T., Bhattacharya S., Akande V.A. on behalf of the Royal College of Obstetricians and Gynaecologists. Multiple Pregnanci es Following Assisted Conception. Scientific Impact Paper. 2018: 22.
14. Wang X., Du M., Guan Y.et al. Comparative neonatal outcomes in singleton births from blastocyst transfers or cleavage-stage embryo transfers: a systematic review and meta-analysis. Reprod Biol Endocrinol. 2017;15(1):36. doi: 10.1186/s12958-017- 0255-4.
15. Dar S., Librach C.K., Gunby J. et al. Increased risk of preterm birth in singleton pregnancies after blastocyst versus Day 3 embryo transfer: Canadian ART Register (CARTR) analysis. Human Reproduction. 2013; 28(4):924–928.
16. Mascarenhas M., Sunkara S.K., Antonisamy B. et al. Higher risk of preterm birth and low birth weight following oocyte donation: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2017;218:60-67. doi: 10.1016/j.ejogrb.2017.09.015.

ORCID and contributionship:

Tamara G. Romanenko: 0000-0003-0157-6223 [A,E,F]
Olha M. Sulimenko: 0000-0003-1948-8387 [A,B,C,D]
Svitlana O. Ovcharenko: 0000-0002-2493-7741 [C,D]

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Tamara G. Romanenko
Shupyk National Medical Academy
of Postgraduate Education
of the Ministry of Health of Ukraine
9 Dorohozhytska St., 04112 Kyiv, Ukraine
tel: +380677219619,
e-mail: romanenko.tmr@gmail.com

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

ORIGINAL ARTICLE

SEASONAL FACTOR AND FREQUENCY OF DETECTION OF HERPES VIRUSES AND INFLUENZA VIRUS IN PATIENTS WITH ISCHEMIC STROKE

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Tetyana M. Cherenko, Natalia S. Turchyna, Yuliya L. Heletiuk, Natalia G. Andriushkova, Olena V. Kuzminska
O. O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

The aim: To determine the frequency of HSV1, HSV2, VZV, CMV, EBV, HHV6 and influenza virus detection in patients with ischemic stroke in different seasons.

Materials and methods: 144 patients with ischemic stroke were examined: 78 (54.2%) women and 66 (45.8%) men, mean age of 63.1 ± 0.8 years. Detection of the herpesvirus DNA and the influenza virus RNA was performed using PCR monthly in 12 patients.

Results: A manifestation of a viral infection was detected in 32 (22.2%) and virus genomes were observed in 29 (90.6%) patients. Viral infection frequency is significantly lower in summer, compared to winter-autumn; p=0.033. HSV1 and HHV6 were the most common (19 (52.8%) and 16 (44.4%)); VZV was the least common (5 (13.9%)). Influenza virus RNA was detected in 10 (27.8%) patients. In winter-autumn the frequency of HSV1, HSV2, HHV6 viruses detection is significantly higher, compared to the spring-summer ($p<0.05$), and the difference is almost significant for the influenza virus (p=0.060) and the EBV (p=0.060). Association of stroke occurrence with the presence of two or more types of viruses is more common in winter, compared to the summer season: 11 (30.6%) vs. 3 (8.3%), p=0.017.

Conclusions: Prevention and treatment of herpesvirus infections exacerbations, in particular HSV1 and HSV2, which significantly increase in winter, compared to summer, is an important direction of stroke prevention measures in risk groups.

KEY WORDS: ischemic stroke, herpes viruses, influenza virus, seasonal differences

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INTRODUCTION

World stroke statistics remains disappointing due to high morbidity, mortality and disability of the population, and this major problem of both social and economical importance stimulates the continuation of developments, such as measures to prevent acute cerebral pathology [1]. An important direction of research is a study of environmental impact, particularly seasonal fluctuations, on the occurrence of stroke, which becomes particularly important in the context of current climate changes.

Seasonal variability of the ischemic stroke frequency has been shown by numerous studies. However, the conclusions of these scientific works significantly differ. Some authors find a higher incidence of ischemic stroke (IS) in winter [2-4], others – in spring [5, 6], and some studies did not establish a correlation between the season and the incidence of IS at all [1, 7].

A recent meta-analysis showed that existing differences are largely related to different characteristics of the local climate [8]. However, in 29 of the 33 studies included in the meta-analysis, summer was the season with the lowest statistical incidence of IS. When grouped by Köppen climate classification [9] in areas with warm temperate climate, which is typical for most of Eastern European territory and, therefore, Ukraine, the incidence of IS was observed with the highest frequency in winter months, with a subsequent decrease in frequency in spring, autumn and summer.

Seasonal differences in the incidence of stroke are associated with various physical natural factors, such as changes in the amount of sunlight [10, 11], temperature [12, 13], and humidity [14, 15, 16]. Many previous studies suggest that the winter season and low-temperature weather conditions may be responsible for the pathophysiological changes associated with ischemic stroke. In particular, it was shown that hemodynamic factors and indicators of coagulation hemostasis, such as blood pressure, erythrocyte count, platelet count and function, fibrinogen concentration in blood plasma, and inflammatory markers may increase due to increased activity of the sympathetic nervous system associated with low temperatures [4, 17].

Issues related to the increase of serum lipids and blood glucose are discussed to explain the seasonality of acute vascular diseases [18]. Some studies have found an abrupt increase in total cholesterol and triglycerides in the autumn months [19].

A certain role in IS incidence increase in the cold months is assigned to infection factors, including respiratory infections and influenza, which are activated due to reduced immunity caused by the deficiencies of sunlight, vitamins C and D, seasonal depression, etc. [11, 12, 19, 20, 21]. How-

Table I. Clinical and demographic features of the patients.

Indicators	Total (n=144)	Winter (n=36)	Spring (n=36)	Summer (n=36)	Autumn (n=36)		p
Sex M/F (%) 45.8/54,2		47.2/52.8	44.4/55.6	41.7/58.3	44.4/55.6	0.80	
Age, year (M±m) 63.1±0.8		65.9±1.3	60.1±1.7	62.1±1.8	64.4±1.7	>0.05*	
NIHSS, point (M±m) 11.1±0.1		10.9±0.3	10.7±0.2	11.4±0.3	11.2±0.2	>0.05*	
Pathogenetic subtype of stroke							
Atherotrombotic (%)	50.7	50.0	44.4	50.0	58.3		0.866
Cardioembolic (%)	33.3	33.3	41.7	30.6	27.8		
Lacunar (%)	16.0	16.7	13.9	16.4	13.9		
Vascular areas							
Left middle cerebral artery (%)	43.1	41.7	47.2	44.4	38.9		0.839
Right middle cerebral artery (%)	31.9	33.3	36.1	30.6	27.8		
Vertebrobasilar arteries (%)	25.9	25.0	16.7	25.0	33.3		

Notes:*statistically unreliable for all pairwise comparisons

ever, the incidence of viral manifestation and persistence of viruses in patients with IS in different seasons has not been studied.

THE AIM

The goal of this study was to determine the frequency of HSV1, HSV2, VZV, CMV, EBV, HHV6 and influenza virus detection in patients with ischemic stroke in different seasons.

MATERIALS AND METHODS

The study took place over the course of one year from 01.01.2017 to 31.12.2017 at Oleksandrivska Hospital and Kyiv City Clinical Hospital №4 departments. The study was conducted in accordance with the requirements of Good Clinical Practice standards. A total of 144 patients with ischemic stroke were included in the study: 78 (54.2%) women and 66 (45.8%) men. The mean age of patients was 63.1 ± 0.8 years (from 41 years to 81 years).

During each season 36 patients hospitalized by ambulance were examined for the presence of viruses; specifically, 12 patients per month (3 patients per week) in order of their admission to the hospital and if the following inclusion criteria were met:

- a primary ischemic stroke confirmed by Magnetic Resonance Imaging / Computed Tomography;
- a neurological score of 8–16 points according to NIHSS [22]
- one of the following pathogenetic subtypes according to TOAST criteria [23]: atherothrombotic (AT), cardioembolic (CE), lacunar.

The patients were tested for herpes viruses (HSV1, HSV2, VZV, CMV, EBV, HHV6) and influenza virus.

There were following exclusion criteria: recurrent stroke, inability to collect a patient's history, NIHSS score above 17, indeterminate pathogenetic type of stroke, and a lack of informed consent for virological examination.

All patients were questioned on the subject of viral infection manifestation in their medical history (in the 2 weeks prior to stroke). The manifestations were evaluated using the clinical indicators of respiratory disease (rhinorrhea or rhinitis, fever and increased temperature, headache, catarrhal signs in upper respiratory tract, and a herpetic rash around lips and nose).

Detection of herpes virus deoxyribonucleic acid (DNA) and influenza virus ribonucleic acid (RNA) was performed by the means of polymerase chain reaction (PCR) monthly in 12 patients who were hospitalized by ambulance. The DNA of herpes viruses from cells was isolated using a set of reagents DNA-sorb-B DNA kit (AmpliSens, Russia) or "innuPREP Virus DNA Kit" (Analytik Jena AC, Germany) according to the manufacturer's instructions. The DNA concentration was determined spectrophotometrically using a Biophotometer (Eppendorf, Germany). The DNA detection was performed by the means of semi-quantitative PCR, using a set of"AmpliSens®" (AmpliSens, Russia) reagents, according to the manufacturer's recommendations. Each sample that was analyzed by PCR contained 50 nanograms of DNA. The amplification products of GeneRuler ™ DNA Ladder Mix (Fermentas, Lithuania) were analyzed in a 1.7% agarose gel containing 0.01% ethidium bromide. Digital images of PCR products were obtained in UV light of a transilluminator using a Canon Digital IXUS 80IS camera. Analysis of digital images was performed using Gel Imager software (DNA-technology, Russia). In addition, RT-PCR was performed using the AmpliSens kit (AmpliSens, Russia) and EBARPOL (NPF. Litech, Russia), according to the manufacturer's recommendations (qTOWER 2.2 amplifier, Germany).

Detection of influenza virus RNA was performed by the means of PCR. Influenza virus RNA was isolated from cells using reagents kit in real-time (Real-Time RT-PCR kit),

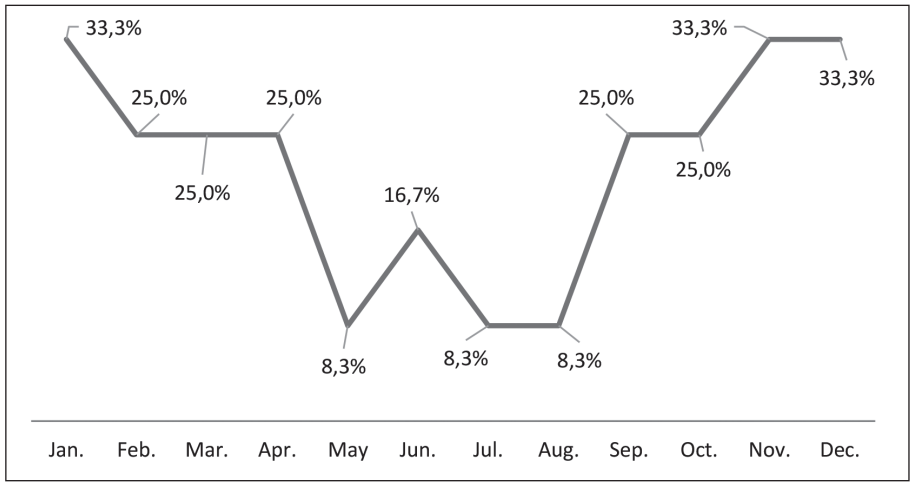

Fig. 1. Distribution of patients by frequency of viral infection by a month of year.

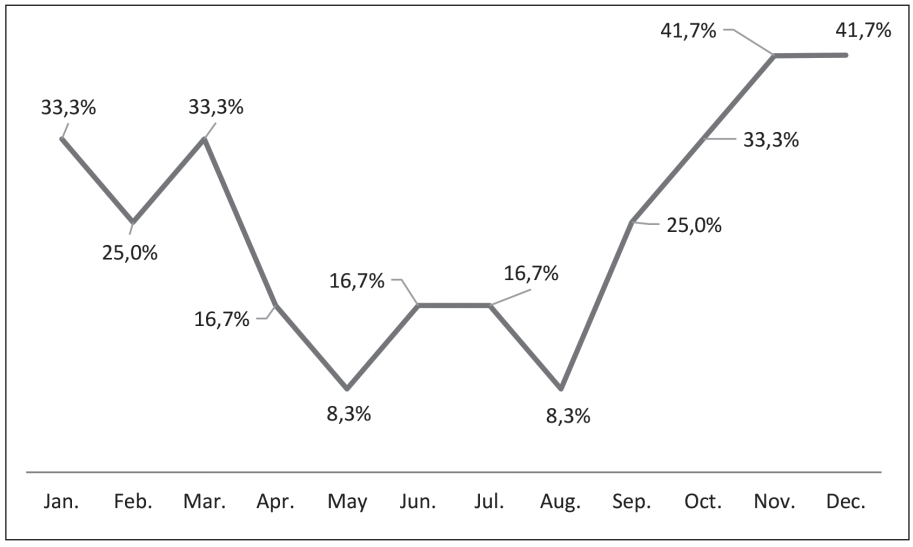

Fig. 2. Distribution of patients by frequency of viral infection by months of the year.

using Real-Time RT-PCR analysis, dPB2-Probe, dPB2-F2 and dPB2-R1 primers.

Statistical processing of the results was performed using the statistical analysis program IBM SPSS Statistics Base v.22. Descriptive statistics were used; comparisons of two independent groups by their average values were performed using the Mann-Whitney U-test, and on a qualitative basis – using Pearson's $\chi 2$. The null hypothesis regarding the equality of variables was rejected at $p < 0.05$.

RESULTS

The overall ratio of men and women was 45.8/54.2, respectively, and did not differ between seasons, p = 0.80 (Table I). The mean age of patients also did not differ significantly from season to season, p> 0,05.

Atherothrombotic subtype of stroke occurred in 73 (50.7% of patients), cardioembolic– in 48 (33.3%), lacunar – in 23 (16.0%) of patients. The distribution of patients by stroke subtype did not statistically differ between seasons (p = 0.886), although there was a tendency for an increase in the percentage of AT subtype in autumn – 58.3%, and CE subtype in spring (41.7%) with an almost identical percentage of AT in these seasons (Table I).

Ischemic stroke in the left middle cerebral artery territory (MCA) occurred in 62 (43.1%) patients; in the right MCA – 46 (31.9%), in the vessels of vertebrobasilar artery territory – 36 (25.0%). The distribution of patients by the territory of the affected vessel also did not significantly differ depending on the season (p = 0.839) (Table I).

A manifestation of a viral infection was found in 32 (22.2%) patients. In winter – in 11 (30.6%) patients, in spring – in 7 (21.9%), in summer – in 4 (11.1%), and in autumn – in 10 (27.8%), p = 0.485. Although overall there were no significant differences in the frequency of viral infection by seasons, at the same time in summer the frequency of viral infection was significantly lower than in winter, p = 0.042, and almost significantly lower than in autumn, p = 0.074.

The distribution of the frequency of viral infection in stroke patients by a month of year showed approximately the same frequency of 33.3% from November to January, with a decrease to 25.0% in February, March, April and September and October, and with the lowest rates in May and in summer months – 8,3% (Fig. 1).

Virus genomes were found in 36 (25%) patients with stroke. In particular, the genomes were found in 29 (90.6%) patients among those who had a viral manifestation (signs

Table II. The types viruses frequency detection and its quantity in the association in different seasons

Indicators	Total (n=144)	Winter n=36	Spring n=36	Summer n=36	Autumn n=36	p
Viruses						
HSV1, n (%)	19 (13.2)	9 (25.0)	3 (8.3)	3 (8.3)	4 (11.1)	0.111
HSV2, n (%)	14 (9.7)	7 (19.4)	3 (8.3)	0 (0.0)	4 (11.1)	**0.048**
VZV, n (%)	5 (3.5)	1 (2.8)	2 (5.6)	1 (2.8)	1 (2.8)	0.891
EBV, n (%)	10 (6.9)	4 (11.1)	2 (5.6)	1 (2.8)	3 (8.3)	0.542
CMV, n (%)	8 (5.6)	3 (8.3)	1 (2.8)	1 (2.8)	3 (8.3)	0.548
HHV6, n (%)	16 (11.1)	7 (19.4)	1 (2.8)	1 (2.8)	7 (19.4)	**0.018**
Flu, n (%)	10 (6.9)	5 (13.9)	2 (5.6)	0 (0.0)	3 (8.3)	0.133
Quantity of viruses in the association						
No viruses, n (%)	108 (75.0)	24 (66.7)	29 (80.8)	31 (84.1)	24 (66.7)	0.131
1, n (%)	8 (22.2)	1 (2.8)	2 (5.6)	2 (5.6)	3 (8.3)	0.787
2, n (%)	17 (47.2)	5 (13.9)	3 (8.3)	3 (8.3)	6 (16.7)	0.616
3, n (%)	6 (16.7)	2 (5.6)	2 (5.6)	0 (0.0)	2 (5.6)	0.237
4, n (%)	3 (8.3)	2 (5.6)	0 (0.0)	0 (0.0)	1 (2.8)	0.133
5, n (%)	2 (5.6)	2(5.6)	0(0.0)	0(0.0)	0(0.0)	0.279

of acute viral infection or exacerbation of latent persistent herpes infection) and in 3 (9.4%) patients with stroke without viral manifestation (p = 0.001) (Table II).

The genomes of viruses in winter were found in 12 (33.3%) patients, in spring – in 7 (19.4%), in summer – in 5 (13.9%), and in autumn – in 12 (33.3%) patients, p = 0.131.

Although in general there were no significant differences in the frequency of viral infection detection by season, at the same time in the summer season the frequency of viral infection detection was significantly lower compared to the winter-autumn period, p = 0.033.

The distribution of the frequency of the viruses genome detection in patients with stroke by months of the year showed approximately the same frequency from October to January – 33.3% – 41.7% (excluding February – 25.0%), with an average of 35.0% and a decrease to 8.3 –25.0% from March through August , an average being 17.9%, p = 0.019 (Fig. 2).

In the virus-positive group, HSV1 was the most common, occurring in 19 (52.8%) patients (13.2% of the total) and HHV6 – in 16 (44.4%) patients (11.1%), with VZV-5 occurring least often (13.9%). Influenza virus RNA was found in 10 (27.8%) patients.

Despite the fact that there was a tendency toward a higher frequency of the viruses genome detection in winter and autumn compared to spring, and, most of all, summer, the differences did not reach statistical significance, p = 0.052. At the same time, there were significantly more patients with two or more viruses in the winter season compared to the summer season: 11 (30.6%) against 3 (8.3%), p = 0.017.

The frequency of viruses detection (except for HSV2 and HHV6) did not significantly differ depending on the season, when comparing the distribution of the frequency of detection of certain types of viruses in different seasons (Table II).

At the same time there is a significantly higher (p <0.05) frequency of HSV1, HSV2, HHV6 viruses detection in the winter-autumn period, compared to the spring-summer period, and an almost significant difference for influenza viruses (p = 0.060) and EBV (p = 0.060).

There was no significant correlation between viral manifestations, the presence of the viruses genome and severe neurological deficits: r = 0.037 and r = 0.039, respectively.

There also was no significant correlation between viral manifestations, the presence of the herpes or influenza viruses genome and the age of patients: r= 0.033 and r = 0.096, respectively.

DISCUSSION

In our research we continued to study the issue of seasonal variability of the ischemic stroke frequency, which has been the subject of a number of foreign authors' studies that in particular at the role of a viral infection as a potential risk factor for increased frequency of IS in winter compared to summer [4, 5, 7]. This applies to countries with a warm continental climate, including Ukraine. [8]. The goal of our study was to determine whether there were any seasonal differences in the frequency of detection of the most common types of viruses – herpes viruses and influenza virus in patients after one stroke with moderate and severe neurological deficits. It should be noted that each season groups with the same number of patients were studied; further, the patients also did not significantly differ in age and gender characteristics. Our attention has been drawn to herpes viruses, because of their high prevalence at 30-40% of the population in developing countries [24], as well as toward the influenza virus, the epidemic outbreaks of which continue to regularly damage the health of the population and the economies of countries around the world.

The genome of herpes viruses and influenza virus was found in a total of 36 (25%) patients with stroke. The most prevalent was HSV1 found in 13.2% of patients and HHV6 found in 11.1% of patients; influenza virus was discovered in 6.9% of patients in the pre-stroke period. In most cases (77.8%) viral pathogens were in associations (2, 3, rarely 4 – 5 viruses), p <0,01.

The study has demonstrated a significantly higher percentage of patients with herpesviruses persistence, specifically, with two or more viruses, in winter, compared to the summer season. Thus, according to the literature, a more frequent development of strokes in the winter season (compared to summer in our climate zone), is associated, according to our findings, with a higher frequency of detection of herpes and influenza viruses genomes in patients' blood during the winter season.

It is known that infection and stroke have common pathogenetic pathways and mechanisms: inflammation, hypercoagulation and thrombosis [25]. Therefore, it is logical to assume the existence of an indirect connection between an increase in the frequency of strokes and the increase in the frequency of detection of viral factors that activate during the winter season –a period of reduced immune activity due to a deficiency of sunlight, vitamins D and C, seasonal depression, cold sympathetic systems activation, etc. The obtained data may help to improve the prevention of ischemic stroke by conducting a virological examination in instances of respiratory viral infection manifestation in patients in stroke risk groups in the autumn-winter period, as well as by an active use of influenza vaccinations.

CONCLUSIONS

Detection of the herpes viruses genome in the blood of patients with ischemic stroke with moderate to severe neurological deficits, both with and without the clinical manifestations of viral infection, is more common in winter and autumn.

During the winter-autumn period there is a significantly higher frequency of HSV1, HSV2, HHV6 viruses detection compared to the spring-summer season (p<0,05).

In the winter season, the frequency of patients with two or more types of viruses in an association is significantly higher compared to the summer season: 11 (30.6%) against 3 (8.3%), p = 0.017.

The frequency of herpes viruses detection does not correlate with the age of patients and the severity of neurological deficits: r = 0,096 and r = 0,039, respectively.

REFERENCES

1. Feigin V.L. et al. Global burden of stroke and risk factors in 188 countries, during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2016;15(9):913-924.
2. Jakov Ijevic D., Salomaa V., Sivenius J. et al. Seasonal variation in the occurrence of stroke in a Finnish adult population. The FINMONICA Stroke Register. Finnish monitoring trends and determinants in cardiovascular disease. Stroke. 1996;27(10):1774–9.
3. Wang H., Sekine M., Chen X., Kagamimori S. A study of weekly and seasonal variation of stroke onset. Int J Biometeorol. 2002;47(1):13–20. doi:10.1007/s00484-002-0147-x.
4. Palm F., Dos Santos M., Urbanek C. et al. Stroke seasonality associations with subtype, etiology and laboratory results in the Ludwigshafen Stroke Study (LuSSt). Eur J Epidemiol. 2013;28(5):373-81. doi: 10.1007/s10654-013-9772-4.
5. Karagiannis A., Tziomalos K., Mikhailidis P. D. et al. Seasonal variation in the occurrence of stroke in Northern Greece: A 10 year study in 8204 patients. Neurological Research. 2010; 32(3): 326–331. doi.org/10.1179/174313208X331608.
6. Mao Y., Schnytzer Y., Busija L. et al. "MOONSTROKE": Lunar patterns of stroke occurrence combined with circadian and seasonal rhythmicity-a hospital based study. Chronobiology International. 2015;32(7): 881–888. doi.org/10.3109/ 07420528.2015.1049614.
7. Jakovljevic´ D., Salomaa V., Sivenius J. et al. Seasonal variation in the occurrence of stroke in a Finnish adult population. Stroke. 1996; 27(10):1774–1779.
8. Li Y., Zhou Z., Chen N. et al. Seasonal variation in the occurrence of ischemic stroke: A meta-analysis. Environ Geochem Health. 2019;41(5):2113-2130. doi: 10.1007/s10653-019-00265-y.
9. McKnight T.L., Hess D. Climate Zones and Types: The Köppen System // Physical Geography: A Landscape Appreciation. NJ: Prentice Hall. 2000: 200—201.
10. Kent S. T., McClure L. A., Jud, S. E. et al. Short- and long term sunlight radiation and stroke incidence. Annals of Neurology. 2013; 73(1), 32–37. https://doi.org/10.1002/ana. 23737.
11. Iwamoto J., Takeda T., Matsumoto H. Sunlight exposure is important for preventing hip fractures in patients with Alzheimer's disease, Parkinson's disease, or stroke. Acta Neurologica Scandinavica. 2012; 125(4): 279–284. https://doi.org/10.1111/j.1600-0404.2011.01555.x.
12. Lichtman J.H., Leifheit-Limson E.C., Jones S.B. et al. Average temperature, diurnal temperature variation, and stroke hospitalizations. Journal of Stroke and Cerebrovascular Diseases. 2016; 25(6): 1489–1494. https://doi.org/10.1016/j.jstrokecerebrovasdis. 2016.02.037.
13. Chen G., Zhang Y., Song G. et al. Is diurnal temperature range a risk factor for acute stroke death? International Journal of Cardiology. 2007; 116(3): 408–409. https://doi.org/10.1016/j.ijcard.2006.03.
14. Lai P. M., Dasenbrock H., Du R. The association between meteorological parameters and aneurysmal subarachnoid hemorrhage: A nationwide analysis. PLoS ONE. 2014 ; 9(11): e112961. https://doi.org/10.1371/journal.pone. 0112961.
15. Mostofsky E., Wilker E. H., Schwartz J. et al. Short-term changes in ambient temperature and risk of ischemic stroke. Cerebrovascular Diseases Extra. 2014; 4(1): 9–18. https://doi.org/10. 1159/000357352.
16. Hori A., Hashizume M., Tsuda Y. et al. Effects of weather variability and air pollutants on emergency admissions for cardiovascular and cerebrovascular diseases. International Journal of Environmental Health Research. 2012; 22(5): 416–430. https:// doi.org/10.1080/09603123.2011.650155.
17. Shinkawa A., Ueda K., Hasuo Y. et al. Seasonal variation in stroke incidence in Hisayama, Japan. Stroke. 1990; 21: 1262–1267.
18. Hopstock L. A., Barnett A. G., Bonaa K. H. et al. Seasonal variation in cardiovascular disease risk factors in a subarctic population: The Tromso Study 1979–2008. Journal of Epidemiology and Community Health. 2013;67(2):113–118. https://doi. org/10.1136/jech-2012-201547.
19. Tofler G.H., Muller J.E. Triggering of acute cardiovascular disease and potential preventive strategies. Circulation. 2006; 114(17): 1863–72. doi:10.1161/CIRCULATIONAHA.105.596189.

20. Warren-Gash C., Blackburn R., Whitaker H. et al. Laboratory-confirmed respiratory infections as triggers for acute myocardial infarction and stroke: a self-controlled case series analysis of national linked datasets from Scotland.Eur Respir J. 2018;51(3):1701794. doi: 10.1183/13993003.01794-2017.
21. Lin L.Y., Bhate K., Forbes H. et al. Vitamin D deficiency or supplementation and the risk of human herpesvirus infections or reactivation: a systematic review protocol. BMJ Open. 2019;9(10):e031867. doi: 10.1136/bmjopen-2019-031867.
22. Odderson I.R. The National Institutes of Health Stroke Scale and its importance in acute stroke management. Phys Med Rehabil Clin N Am. 1999: 10(4):787-800.
23. Kolominsky-Rabas P.L., Weber M., Gefeller O. et al. Epidemiology of ischemic stroke subtypes according to TOAST criteria. Incidence, recurrence, and long term survival in ischemic stroke subtypes: A population-based study. Stroke. 2001; 32:2735–2740.
24. Esenwa C.C., Elkind M.S. Inflammatory risk factors, biomarkers and associated therapy in ischaemic stroke. Nature Reviews Neurology. 2016. 12:594–604.
25. Forbes I.D., Harriet J., Williamson E. et al. Association of herpesviruses and stroke: Systematic review and meta-analysis PLoS One. 2018;13(11):e0206163. doi: 10.1371/journal.pone.0206163.

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ORCID and contributionship:
Tetyana M. Cherenko: 0000-0003-0867-5929 [A,C,D,E,F]
Natalia S. Turchyna: 0000-0002-9992-4929 [A,B,C,D]
Yuliya L. Heletiuk: 0000-0002-7260-6496 [A,D]
Natalia G. Andriushkova: 0000-0001-9318-8978 [B]
Olena V. Kuzminska: 0000-0002-8259-1611 [E]

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CORRESPONDING AUTHOR
Tetyana M. Cherenko
O. O. Bogomolets National Medical University
13 Tarasa Shevchenko blvd, 01601, Kyiv, Ukraine
tel: +380677121065
e-mail: tcherenko@ukr.net

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ORIGINAL ARTICLE

TUMORS AND TUMOR-LIKE LESIONS OF THE SALIVARY GLANDS: MORPHOLOGICAL CHARACTERISTICS OF THE SURGICAL MATERIAL

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Igor S. Brodetskyi[1], Vladislav A. Malanchuk[1], Bogdan V. Sorokin[2], Mykhailo S. Myroshnychenko[3], Yuliya I. Beketova[2], Olena O. Dyadyk[2], Nataliia V. Kapustnyk[3], Mykhailo S. Krotevych[4], Sergey B. Brodetskyi[5]

[1]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[2]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[3]KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE
[4]NATIONAL CANCER INSTITUTE, KYIV, UKRAINE
[5]TARAS SHEVCHENKO NATIONAL UNIVERSITY OF KYIV, KYIV, UKRAINE

ABSTRACT

The aim is to give a morphological characteristic of tumors and tumor-like lesions of the salivary glands in the course of a comprehensive analysis of the surgical material.

Materials and methods: The study used surgical material from 67 patients with various pathologies of the salivary glands. The patients were treated at Kyiv City Clinical Hospital No. 12 (Ukraine, Kiev) from 2014 to 2018. The resulting material was fixed in a 10% solution of neutral formalin (pH 7.4) for 24-48 hours, carried out according to the generally accepted technique and embedded in paraffin. Serial sections 2 μm thick were made from paraffin blocks and stained with hematoxylin and eosin. Microspecimens were studied, using Olympus BX-41 microscope (Japan).

Results: A comprehensive morphological study of the surgical material of patients with salivary gland pathology undergoing treatment at Kyiv City Clinical Hospital No. 12 (Ukraine, Kiev) for the period from 2014 to 2018 revealed benign tumors and tumor-like lesions of these glands. Pleomorphic adenomas and adenolymphomas represented benign tumors of the salivary glands, while cysts represented tumor-like lesions.

Different ratios of parenchymal and stromal components characterized pleomorphic adenomas of the salivary glands. On this basis, we identified three tumor variants (mesenchymal (15 cases, 50.0 %); mixed or classic (10 cases, 33.3 %); epithelial (5 cases, 16.7 %)). A characteristic microscopic feature of salivary gland adenolymphomas was a pronounced predominance of the parenchyma over the stroma. A well-defined wall, represented by connective tissue fibers and epithelial lining, characterized the cysts of the salivary glands. The connective tissue capsule separating the tumor tissue from the intact tissue of the salivary gland was clearly pronounced in adenolymphomas, and in pleomorphic adenomas it could be clearly expressed, intact, with tumor invasion or absent. In mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant, there was a frequent tumor invasion of the capsule, thinning of the capsule or its absence.

Conclusions: Morphological examination of the surgical material allows us to diagnose tumors (pleomorphic adenomas and adenolymphomas) and non-tumor lesions (cysts) of the salivary glands. This, of course, is of great therapeutic, diagnostic and prognostic value. Among the identified pathology of the salivary glands, pleomorphic adenomas cause certain difficulties in morphological diagnosis due to their structural diversity and heterogeneity, sometimes requiring morphometric, immunohistochemical or genetic research.

KEY WORDS: tumors, tumor-like lesions, salivary glands, surgical materials, morphology

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INTRODUCTION

Saliva, produced by salivary glands, is a complex mixture of fluid, electrolytes, enzymes, and macromolecules. They function together and perform several important roles: lubrication to aid in swallowing and digestion; digestion of starches with salivary amylase; modulation of taste; protection against dental caries; and defense against pathogens [1]. The salivary glands are the paired parotid, submandibular and lingual glands alongside several hundred minor salivary glands, distributed through the upper aerodigestive system [2].

Many diseases, both local and systemic, can affect the salivary glands. Prevailing salivary gland diseases depend on various etiological factors. Small stones formed in the gland ducts can block the glands. Viral, bacterial, or (rarely) fungal agents may infect the glands or they may be the targets of autoimmune attacks, affecting their functions. In the salivary glands, benign and malignant tumors can develop [3]. Tumor-like lesions can also develop in the salivary glands [4].

At present, there are special methods diagnosing diseases of the salivary glands along with the questioning of the patient, examination and palpation of the affected area. These are: echography, thermal visiography, sialometry, scintigraphy and radionuclide scanning, radiography of the salivary gland, using radiopaque substances, and computed

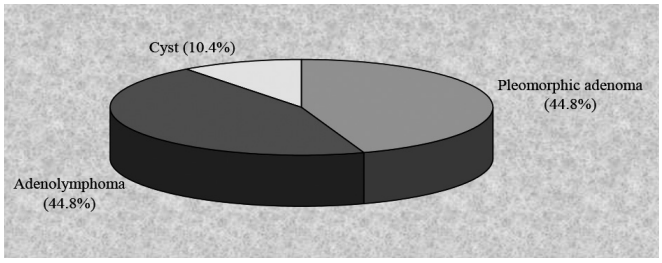

Fig. 1. The survey microscopy results of the studied cases.

tomography [5]. Despite the available arsenal of methods for diagnosing pathology of the salivary glands, the percentage of diagnostic errors, according to our data and the data of other scientists, varies from 46 % to 70 % [6].

In patients with pathology of the salivary glands the choice of treatment method, planning of the nature and volume of surgical intervention in most cases depends on the results of morphological examination.

THE AIM

The aim is to give a morphological characteristic of tumors and tumor-like lesions of the salivary glands in the course of a comprehensive analysis of the surgical material.

MATERIALS AND METHODS

The study used surgical material from 67 patients with various pathologies of the salivary glands. The patients were treated at Kyiv City Clinical Hospital No. 12 (Ukraine, Kiev) from 2014 to 2018. The resulting material was fixed in a 10% solution of neutral formalin (pH 7.4) for 24-48 hours, carried out according to the generally accepted technique and embedded in paraffin. Serial sections 2 μm thick were made from paraffin blocks and stained with hematoxylin and eosin. Microspecimens were studied, using Olympus BX-41 microscope (Japan).

RESULTS AND DISCUSSION

Diagnosis of tumor and non-tumor diseases of the salivary glands is sometimes difficult and requires an interdisciplinary approach. Morphological examination of the salivary gland has an important therapeutic, diagnostic and prognostic value.

Detailed survey microscopy of all studied cases (fig. 1) in 60 cases (89.6 %) revealed benign tumors of the salivary glands, represented by pleomorphic adenoma (30 cases, 44.8 %) and adenolymphoma (30 cases, 44.8 %). Cysts of the salivary glands were diagnosed in 7 cases (10.4 %).

In our earlier research, we identified morphological features of all cases of pleomorphic adenomas [7]. It was noted that these tumors were characterized by structural diversity and heterogeneity, different ratios of parenchymal (epithelial) and mesenchymal (stromal) components. Based on this, we identified three histological variants of the tumor. In 15 cases (50.0 %), a mesenchymal variant was found, characterized by a predominance of the stroma over the parenchyma (fig. 2). Mixed or classic variant of the tumor was identified in 10 cases (33.3 %), characterized by an almost equal content of stroma and parenchyma. An epithelial variant was determined in 5 cases (16.7 %) with prevalence of parenchyma over the stroma (fig. 3).

In pleomorphic adenomas, epithelial and myoepithelial cells represented the parenchymal component. Epithelial cells were of various sizes and shapes (round, polygonal, cubic). In part of the visual fields, these cells were of the basaloid, spindle-cell, squamous, clear-cell, or plasmacytoid types. The epithelial component less commonly included mucous, sebaceous, serous or multinucleated cells. Accumulations of epithelial cells more often formed nests and cords, which anastomosed with each other, and less often – solid, trabecular, cystic, glandular, ductal or tubular structures.

We did not find myoepithelial cells in all studied cases, as they were focally located. In some places, these cells were tightly placed to each other, and in some places they were loose, had a polygonal or fusiform shape. In part of the visual fields, they resembled smooth muscle cells.

The stroma of pleomorphic adenomas showed connective tissue fibers, and vessels of various sizes were located between them. Moderate hemodynamic disturbances were revealed in the stroma of some tumors, characterized by edema, vascular congestion, and small focal diapedetic hemorrhages in the perivascular space. In all cases, we observed myxoid, chondroid and mucoid zones in the stroma of the tumor, while in isolated cases – osteoid zones and areas of lipomatosis.

Slow growth, scanty symptoms and a tendency to recurrence characterize pleomorphic adenoma of the salivary glands [8].

Recurrence of pleomorphic adenomas, according to many scientists, directly depends on the morphological state of the tumor capsule [9]. With the mesenchymal variant of the tumor, the capsule in 3 cases (20.0 %) was intact, in 4 cases (26.7 %) it was not detected, and in 8 cases (53.3 %) we noticed invasion of the tumor into the capsule. The tumor capsule in the mixed variant in 2 cases (20.0 %) was intact, in 4 cases (40.0 %) it was not detected, in 4 cases (40.0 %) it was damaged due to tumor invasion. In the epithelial variant, the capsule in 1 case (20.0 %) was with tumor invasion, and in 4 cases (80.0 %) it looked intact.

Scientists also noted that the capsule of pleomorphic adenomas had thick, dense, fibrous tissue. In some cases, it was discontinuous, or absent or became invaded and even penetrated by a tumor. It was identified the correlation between capsular features and parenchymal/stromal ratio of the tumor. In series of cases hypercellular pleomorphic adenomas had a thick capsule while hypocellular tumors had a thin capsule [10].

Other researches emphasized absence of association between capsular features and histological subtype of pleomorphic adenomas [11].

Microscopically, in 30 cases, adenolymphoma of the salivary glands showed glandular and cystic cavities lined

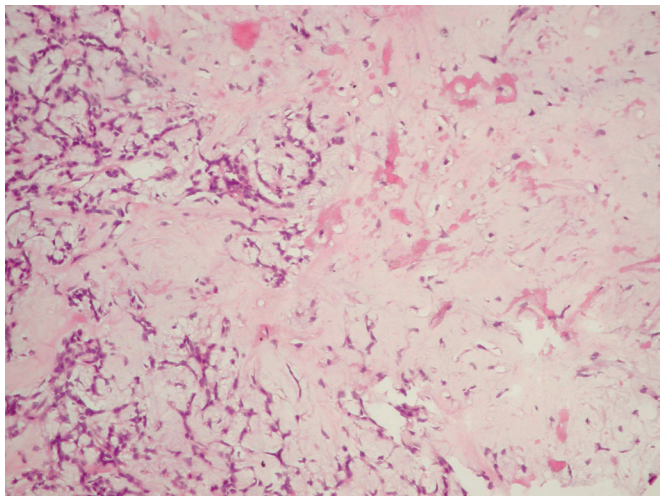

Fig. 2. Mesenchymal variant of pleomorphic adenoma. Hematoxylin and eosin staining, × 200.

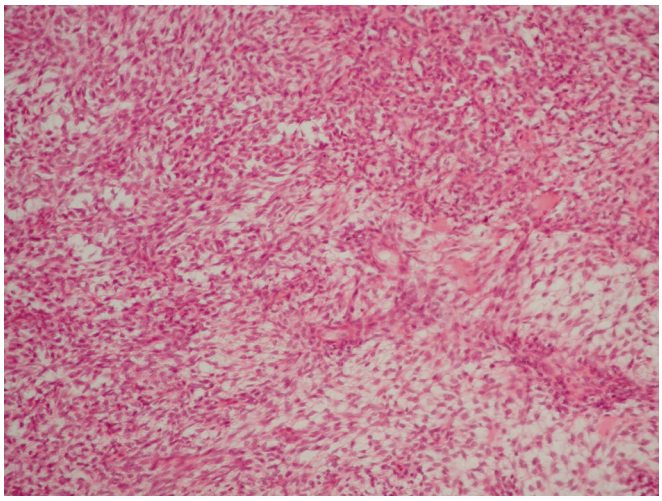

Fig. 3. Epithelial variant of pleomorphic adenoma. Hematoxylin and eosin staining, × 200.

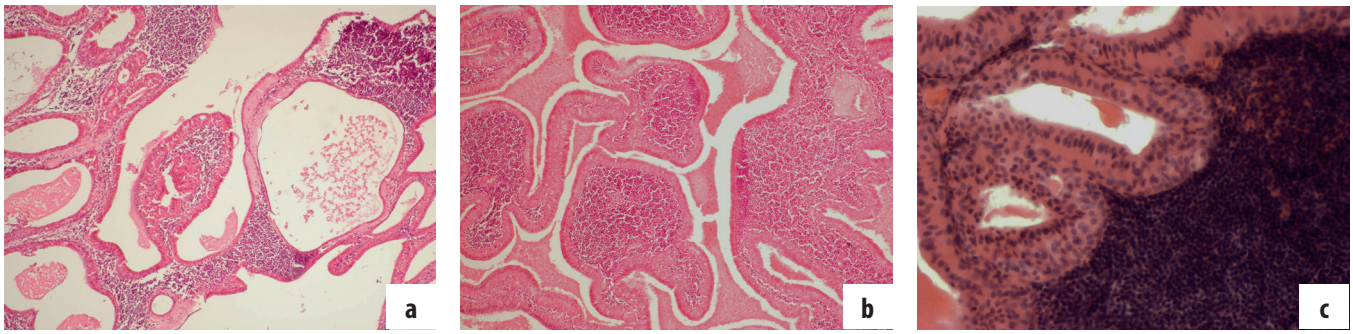

Fig. 4. Microscopic structure of adenolymphoma. Hematoxylin and eosin staining, a) × 100, b) × 100, c) × 400.

with two-row epithelium (fig. 4). In all cases, we noticed the formation of papillary structures, protruded into the lumen of the cystic cavities. Eosinophilic contents were in the lumens of glandular and cystic-papillary structures. In 24 cases (80.0 %), eosinophilic contents with dystrophic altered epithelial cells were in the lumens of the cystic cavities. In 3 cases (10.0 %), the epithelial lining was not detected in some of the cystic cavities. In 1 case (3.3 %), an increase in the number of layers was determined in the epithelial layer, as a result of which it lost its characteristic two-layer structure. The tumor stroma had connective tissue fibers, vessels, pronounced lymphoid infiltration, which in a significant number of visual fields formed small and large lymphoid follicles with light centers. In all studied cases of the salivary glands adenolymphoma we noted, firstly, a pronounced predominance of the parenchymal component over the stromal one; secondly, a clear connective tissue capsule separating the tumor tissue from the intact tissue.

Recurrences of adenolymphomas of the salivary glands, according to the literature, are very rare and occur in connection with the multicentric growth of this tumor. Malignancy with adenolymphoma is also extremely rare [12].

Thus, in this study, benign tumors of the salivary glands represented by various histological variants of pleomorphic adenomas and adenolymphomas. The works of many scientists highlight, among all benign tumors of the salivary glands, a pronounced predominance of cases with pleomorphic adenomas and adenolymphomas [8].

Histogenesis of pleomorphic adenomas and adenolymphomas of the salivary glands is a controversial issue. The source of developing pleomorphic adenomas of the salivary glands can be epithelial cells lining the secretory sections and excretory ducts; myoepithelial cells containing secretory elements in the cytoplasm; stroma of the tumor [13, 14].

Many scientists associate the origin of adenolymphoma with the ducts of salivary glands epithelium. The lymphoid component of adenolymphoma derives from altered lymph nodes, or occurs secondarily due to a lesion in the epithelium, or a combination of both. Aside from B- and T-cells, the infiltrate also contains macrophages and mast cells, suggesting local presence of an antigen triggering immune cell recruitment [15].

Survey microscopy revealed cysts of the salivary glands in 7 cases, which, as is known, belong to tumor-like lesions [4].

In all the cases studied, the cyst wall of the salivary glands was represented by connective tissue fibers and epithelial lining (fig. 5, 6), which was thinned or not visible in single visual fields. In the cyst walls in 6 cases (85.7 %) there was a moderately pronounced diffuse lymphoid-histiocytic infiltration, combined in 2 cases (28.6 %) with a few neutrophilic leukocytes. In 5 cases (71.4 %) moderate hemodynamic

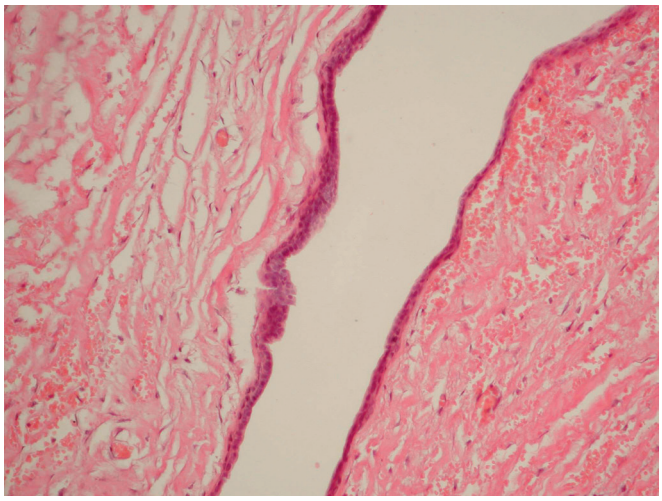

Fig. 5. The wall of the cyst of the salivary gland, represented by connective tissue fibers and epithelial lining. Moderate hemodynamic disturbances in the cyst wall. Hematoxylin and eosin staining, × 200.

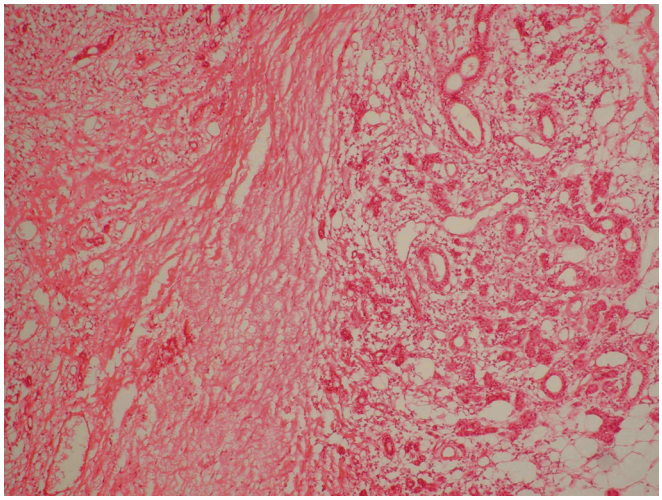

Fig. 6. Lymphoid-histiocytic infiltration with an admixture of a few neutrophilic leukocytes in the cyst wall and adjacent tissue of the salivary gland. Atrophic and sclerotic changes in the salivary gland tissue. Hematoxylin and eosin staining, × 200.

disturbances were revealed, represented by edema, vascular congestion and small focal hemorrhages (fig. 5).

All cases revealed sclerotic changes with atrophy of the parenchymal component in the tissue of the salivary gland adjacent to the cysts. They combined with moderate diffuse lymphoid-histiocytic infiltration with an admixture of single neutrophilic leukocytes in 2 cases (28.6 %), in 6 cases (85.7 %) with hemodynamic changes, in 1 case (14.3 %) with small calculi in the excretory ducts (fig. 6). Thus, the tissue of the salivary glands adjacent to the cyst wall showed signs of chronic sialoadenitis.

The reasons for the formation of the salivary glands cysts can be obstruction of the salivary gland duct with a stone; compression and narrowing of the duct of the gland from the outside by a tumor or due to the excessive development of connective tissue (sclerosis). The latter, as is known, results from the inflammatory process caused by a damaging factor of various origins (biological, chemical, physical, mechanical, etc.).

Salivary gland cysts can also be of congenital origin. They develop from an accessory rudimentary duct detached during embryogenesis [16].

CONCLUSIONS

1. A comprehensive morphological study of the surgical material of patients with salivary gland pathology undergoing treatment at Kyiv City Clinical Hospital No. 12 (Ukraine, Kiev) for the period from 2014 to 2018 revealed benign tumors and tumor-like lesions of these glands. Pleomorphic adenomas and adenolymphomas represented benign tumors of the salivary glands, while cysts represented tumor-like lesions.
2. Morphological examination of the surgical material allowed us to diagnose tumor and non-tumor diseases of the salivary glands. This, of course, was of great therapeutic, diagnostic and prognostic value. Among the identified pathology of the salivary glands, pleomorphic adenomas caused certain difficulties in morphological diagnosis due to their structural diversity and heterogeneity, sometimes requiring morphometric, immunohistochemical or genetic research.
3. Different ratios of parenchymal and stromal components characterized pleomorphic adenomas of the salivary glands. On this basis, we identified three tumor variants (mesenchymal (15 cases, 50.0 %); mixed or classic (10 cases, 33.3 %); epithelial (5 cases, 16.7 %)). A characteristic microscopic feature of salivary gland adenolymphomas was a pronounced predominance of the parenchyma over the stroma. A well-defined wall, represented by connective tissue fibers and epithelial lining, characterized the cysts of the salivary glands.
4. The connective tissue capsule separating the tumor tissue from the intact tissue of the salivary gland was clearly pronounced in adenolymphomas, and in pleomorphic adenomas it could be clearly expressed, intact, with tumor invasion or absent. In mesenchymal and mixed variants of pleomorphic adenomas, in comparison with the epithelial variant, there was a frequent tumor invasion of the capsule, thinning of the capsule or its absence.

The prospect for further research is to clarify the factors related to the etiopathogenesis of pleomorphic adenomas, adenolymphomas and cysts of the salivary glands may be favorable to the understanding and management of such human illnesses.

REFERENCES

1. Wilson KF, Meier JD, Ward PD. Salivary gland disorders. American Family Physician. 2014;89(11):882-888.
2. Rahayuningtyas ED, Setiadhi R. Virus as a cause of salivary gland diseases. ODONTO: Dental Journal.2019;6(1):37-43.
3. Sisto M. Special issue – «Diseases of the salivary glands». Journal of Clinical Medicine. 2020;9:3886. doi:10.3390/jcm9123886.
4. Kessler AT, Bhatt AA. Review of the major and minor salivary glands, part 2: neoplasms and tumor-like lesions. Journal of Clinical Imaging Science. 2018;8(48). doi: 10.4103/jcis.JCIS_46_18.

5. Osipyan EM, Berezina AE, Galstyan MV, et al. Znachenie citomorfologicheskih issledovanij v diagnostike zabolevanij sljunnyh zhelez. Importance of cytomorphological studies in the diagnosis of salivary gland disorders. Medical News of the North Caucasus. 2011;2:44-48. (Ru).
6. Brodetskyi IS. Analiz klinichnogo ta patogistologichnogo diagnoziv hvoryh z novoutvorennjamy slynnyh zaloz za danymy arhivnyh istorij hvoryh kliniky NMU imeni O.O. Bogomolcja v period 2014-2018 roky. Analysis of clinical and pathohistological diagnoses of patients with neoplasms of the salivary glands according to the archival histories of patients in the clinic of the O.O. Bogomolets NMU in the period 2014-2018 years. Stomatological Bulletin. 2019;31(1):84-87. (Ua).
7. Brodetskyi IS, Dyadyk OO, Myroshnychenko MS, Zaritska VI. Morphological characteristics of pleomorphic adenomas of salivary glands (analysis of the surgical material). Wiadomości Lekarskie.2020;73(11):2339-2344.
8. Speight PM, Barrett AW. Salivary gland tumours: diagnostic challenges and an update on the latest WHO classification. Diagnostic Histopathology. 2020;26(4):147-158.
9. Yaremenko AI, Kutukova SI, Harutyunyan, Petrov NL. Mozhno ly prognozyrovat recydyv pleomorfnoj adenomy? Analyz arhyvnyh dannyh pacyentov s pleomorfnoj adenomoj po dannym klynyky cheljustno-lycevoj hyrurgyy FGBOU VO PSPbGMU ym. Y.P. Pavlova MZ RF. Is it possible to predict the recurrence of pleomorphic adenoma? A systematic review of surgical treatment of patients with pleomorphic adenoma according maxillofacial surgery clinic of Pavlov First Saint-Petersburg State Medical University. Dental Institute. 2019;4:73-75. (Ru).
10. Guerra G, Testa D, Montagnani S, et al. Surgical management of pleomorphic adenoma of parotid gland in elderly patients: role of morphological features. International Journal of Surgery. 2014;12:S12-S16.
11. Lopes MLDd, Barroso KMA, Henriques ÁCG, et al. Pleomorphic adenomas of the salivary glands: retrospective multicentric study of 130 cases with emphasis on histopathological features. European Archives of Oto-rhino-laryngology. 2017;274:543-551.
12. Liang CH, Di WY, Ren JP, et al. Imaging, clinical and pathological features of salivary gland adenolymphoma. European Review for Medical and Pharmacological Sciences. 2014;18:3638-3644.
13. Trandafirescu M, Cotuțiu C, Cojocaru E, Foia L. Immunohistochemical aspects in pleomorphic adenoma, related to its histogenesis and malignization. Romanian Journal of Oral Rehabilitation. 2012;4(4):11-16.
14. Almeslet AS. Pleomorphic adenoma: a systematic review. International Journal of Clinical Pediatric Dentistry. 2020: 10.5005/jp-journals-10005-1776.
15. Porcheri C, Meisel CT, Mitsiadis TA. Molecular and cellular modelling of salivary gland tumors open new landscapes in diagnosis and treatment. Cancers. 2020;12: 3107. doi:10.3390/cancers12113107.
16. Porcheri C, Mitsiadis TA. Physiology, pathology and regeneration of salivary glands. Cells. 2019;8(976). doi:10.3390/cells8090976.

ORCID and contibutionship:
Igor S. Brodetskyi – 0000-0002-9434-4079 [A,D,E,]
Vladislav A. Malanchuk – 0000-0001-8111-0436 [A,F]
Bogdan V. Sorokin – 0000-0003-0511-7550 [B,E]
Mykhailo S. Myroshnychenko – 0000-0002-6920-8374 [B,D]
Yuliya I. Beketova – 0000-0001-8635-1802 [B,C]
Olena O. Dyadyk – 0000-0002-9912-4286 [A,D]
Nataliia V. Kapustnyk – 0000-0002-4875-398X [E,F]
Mykhailo S. Krotevych – 0000-0002-5228-7491 [B,C]
Sergey B. Brodetskyi – 0000-0002-6175-2797 [D,E,F]

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CORRESPONDING AUTHOR
Mykhailo S. Myroshnychenko
Pathological Anatomy Department,
Kharkiv National Medical University
str. Svetlaya 27A, apt. 70, 61129, Kharkiv, Ukraine
tel: +380501699763, +380961033038
e-mail: msmyroshnychenko@ukr.net

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ORIGINAL ARTICLE

SURGICAL SITE INFECTION AFTER CESAREAN SECTION IN UKRAINE: RESULTS A MULTICENTER STUDY

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Aidyn G. Salmanov[1], Alla D. Vitiuk[1], Oleg M. Ishchak[1,2], Kateryna S. Insarova[1], Serhii L. Chyrva[3], Marina L. Kuzomenska[3], Oleg V. Golianovsky[1]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]TERNOPIL PERINATAL CENTER, TERNOPIL, UKRAINE
[3]SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

The aim: To obtain the first national estimates of the current prevalence rate of SSI after CSEC and antimicrobial resistance of causing pathogens in Ukraine.

Materials and methods: We performed a retrospective multhicentre cogort study based surveillance data was conducted from May 2017 to December 2019. The study population consisted of all women who had a cesarean section in 11 regional women hospitals of Ukraine. Definitions of SSI after cesarean section were used from the CDC/ NHSN.

Results: A total of 2 326 CSEC operations and 14.7% SSI were identified within 30 days of the operation. Of these, 44.4% were superficial incisional SSI, 28.9% were deep incisional SSI, and 26.6% were organ/space SSI, 25.7% of which were classed as endometritis. Of all cases 70.5% of infections were detected post discharge. The most commonly identifed pathogen were *Staphylococcus aureus* (23.5%), *Escherichia coli* (20%), Coagulase-negative staphylococci (8.1%), *Enterococcus* spp. (7.7%), *Pseudomonas aeruginosa* (7.7%), *Enterobacter* spp. (6.4%), and *Streptococcus* spp. (5.6%). The overall proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriaceae was 18.3% and of methicillin-resistance in *S. aureus* (MRSA) 13.9%. Resistance to third-generation cephalosporins was observed in 15.2% *E.coli* and 7.9% *Klebsiella pneumoniae* isolates. Carbapenem resistance was identified in 7.3% of *P.aeruginosa* isolates.

Conclusions: The results of this study revealed high rates of SSI after CSEC and most causing pathogens were associated with resistant to antibiotic stranis. This knowledge is essential to develop targeted strategies to surveillance and reduce the incidence of postoperative infections.

KEY WORDS: Surgical site infection, cesarean section, antimicrobial resistance, pathogens, Ukraine

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INTRODUCTION

Cesarean section (CSEC) is one of the most common surgical procedures performed in the both developed and developing countries [1]. Caesarean delivery in the United States accounts for 32% of all births [2]. In 2017, CSEC in the European Union (EU) was performed at least 1.4 million times. Among the EU Member States, caesareans were most frequent in Cyprus (54.8 %), Romania (44.1%), Bulgaria (43.1%), Poland (39.3%) and Hungary (37.3%) [3]. Similarly, high CSEC rates have been reported in the Australia and China, where 32.4% and 41% of births are by CSEC, respectively [4, 5]. CSEC in Ukraine accounts for up to 23% of all births.

As with any surgery, CSEC can be associated with surgical site infections (SSIs). Postpartum SSI after CSEC is a major cause of long hospital stays and there is an increased cost burden on the public healthcare systems as well as to mothers and their families [6, 7]. These infections are associated with high morbidity and mortality [8, 9] in women. Over the past two decades, along with significant improvements in clinical obstetric care in many countries, the incidence rate of SSIs after CSEC remains an important issue.

Current guidelines for management of SSI recommend the use of antibiotics as well for prevention, and as for both treatment postoperative infections. However, the growing antimicrobial resistance is limiting their use. Resistant SSI is becoming more and more pressing for medical specialists a worldwide. In the available literature, studies on antimicrobial resistance of infectious agents of SSIs after CSEC are limited. Monitoring of the prevalence of etiologic agents of SSIs after CSEC and antimicrobial resistance is necessary to enhance our knowledge of its epidemiology.

There is no surveillance system for SSI in Ukraine. Consequently, incidence rates of SSI after CSEC and antimicrobial resistance in Ukraine are currently unknown. This creates problems as well for physicians and as infection control professionals in Ukraine. To our knowledge, the prevalence of and causative agents of most SSIs among female in-patients have not been studies in Ukraine. This was the basis for our study.

THE AIM

The aim of this study was to obtain the first national estimates of the current prevalence rate of SSI after CSEC and antimicrobial resistance of causing pathogens in Ukraine.

MATERIALS AND METHODS

SETTING AND PARTICIPANTS

We performed a retrospective multicentre cohort study was based on surveillance data of SSI after CSEC in women's from

Table I. Distribution of surgical site infections after caesarean section in the participating hospitals

Hospital	No. of operations	No. of SSIs	Incidence of SSIs per 100 operations [95% CI]
A	218	29	13.3 [11.0 – 15.6]
B	211	25	11.8 [9.6 – 14.0]
C	203	22	10.8 [8.6 – 13.0]
D	229	56	24.5 [21.7 – 27.3]
E	208	19	9.1 [7.1 – 11.1]
F	212	29	13.7 [11.3 – 16.1]
G	218	31	14.2 [11.8 – 16.4]
H	227	43	18.9 [16.3 – 21.5]
I	193	22	11.4 [9.2 – 13.6]
J	206	27	13.1 [10.8 – 15.4]
K	201	39	19.4 [16.7 – 22.1[
Total	2326	342	14.7 [14.0 – 15.4]

January 1st, 2017 to December 31st, 2019 in 11 regional (tertiary) women hospitals of Ukraine. The hospitals had 1850 beds. They are similar in terms of medical equipment, staff and number of beds. Women hospitals were required to have at least one full-time infection-control professional and clinical microbiology laboratory. Only hospitals that provided data using the same surveillance option (patient-based) for at least three years were included in the study.

The study population included 2,326 women aged 20-51 years who underwent cesarean delivery from 2017 to 2019 in 11 regional women hospitals in Ukraine. All women who underwent caesarean section within the 30 day follow-up period met the inclusion criteria and were included in the study cohort. All participants were local residents.

DEFINITIONS AND DATA COLLECTION

Diagnosis of SSIs after CSEC was based on criteria from the CDC/NHSN Surveillance Definitions for Specific Types of Infections [10]. In this study, we analyzed the inpatient data and ambulatory medical records to identify SSIs after CSEC procedures. The surveillance protocol and dataset were based on the CDC/NHSN criteria. We collected the data (demographic and clinical data, microbiology, and outcome information from hospital and outpatient records) using structured CDC/NHSN Checklist. Cases of SSIs that met standard case definitions were identified through active follow up during the hospital stay, on return to hospital, and during visits to ambulatory. The surveillance period for the patients after CSEC was 30 days.

MICROBIOLOGICAL METHODS

All samples were obtained from women with clinical symptoms of SSI. Microbial isolates were identified using standard microbiological techniques, including automated microbiology testing (Vitek-2; bioMe´rieux, Marcy l'Etoile, France). Antibiotic susceptibility testing was performed by using the disk diffusion method (Kirby – Bauer antibiotic testing) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Strains in the intermediate range were classified as resistant for data analysis.

ETHICS

The Shupyk National Healtcare University of Ukraine Ethics Committee approved the waiver of informed consent to participate in this study due to its retrospective design. All participants data were anonymised prior to the analysis.

STATISTICAL ANALYSIS

For surgical procedure (CSEC) under surveillance, were calculated the percentage of SSIs per 100 operations: an indicator which includes both SSIs diagnosed during hospital stay and after discharge from the hospital (detected at hospital readmission or by post-discharge surveillance). Prevalence of SSIs after CSEC was reported as the percentage of the total number of women who had cesarean procedure. We analyzed samples from women's in the context of a study about microbiology of SSI after CSEC and antimicrobial resistance of responsible pathogens. The analysis of statistical data was performed using Excel. Comparisons were undertaken using Student's t-test and Fisher's exact test for categorical variables. Statistical significance was defined as $P<0.05$.

RESULTS

PREVALENCE OF SSI

During the study period (2017-2019) applying all methods of detection, a total of 342 SSIs were identified from 2326 cesarean procedures. The prevalence of SSI after CSEC in Ukraine was 14.7% [95% confidence interval

Table II. Characteristics of patients with SSI after cesarean delivery in Ukraine (P < 0.05)

Variables	Number of participants n	SSI[a]		95% CI[b]
		n	%	
Age of participants				
≤20	117	13	11.1	10.4 – 11.7
21–25	276	42	15.2	14.5 – 15.9
26–30	417	53	12.7	12.0 – 13.4
31–35	436	57	13.1	12.4 – 13.8
36–40	408	68	16.7	15.9 – 17.5
41–45	261	35	13.4	12.7 – 14.1
46–50	233	41	17.6	16.8 – 17.6
≥51	178	33	18.5	17,7 – 19.3
Total	2326	342	14,7	14.0 – 15.4

Note
aSSI, Surgical site infection
bCI, confidence interval.

Table III. Distribution of microorganisms identified in SSIs following cesarean section in women hospitals (Ukraine, 2017-2019)

Microorganisms	All isolates (n=534)	Percentages, %
Gram-positive cocci	247	46.3
Staphylococcus aureus	124	23.2
Coagulase-negative staphylococci	43	8.1
Enterococcus species	41	7.7
Streptococcus species	30	5.6
Other Gram-positive cocci	9	1.7
Gram-negative bacilli, Enterobacteriaceae	194	36.3
Escherichia coli	107	20.0
Citrobacter species	7	1.3
Enterobacter species	34	6.4
Klebsiella pneumoniae	19	3.6
Proteus species	16	3.0
Serratia species	4	0.7
Other Enterobacteriaceae	7	1.3
Gram-negative non- fermentative bacilli	73	13.7
Acinetobacter species	21	3.9
Pseudomonas aeruginosa	41	7.7
Stenotrophomonas maltophilia	5	0.9
Other gram-negative non- fermentative bacilli	6	1.1
Anaerobes	15	2.8
Bacteroides species	5	0.9
Other anaerobes	10	1.9
Fungi	**5**	0.9
Candida species	5	0.9

(CI) 14.0-15.4]. Of these, 152 (44.4%) were superficial incisional SSI, 99 (28.9%) were deep incisional SSI, 91 (26.6%) were organ/space SSI, 25.7% of which were classed as endometritis.

Of all cases 29.5% (101/342) of infections were detected in during the admission period and 70.5% (241/342) were detected post discharge. The incidence rates of SSI were 10.7% [95% CI 10.1 – 11.3] after elective cesarean delivery

and 18% [95% CI 17.2 – 18.8] after urgent cesarean section. None of the patients died as a result of SSIs after CSEC procedure. The incidence of SSIs after caesarean section in the participating hospitals varied significantly (Table I).

In this study the median time the duration admission period for all surgical site infections was 7 days and for deep and organ/space infections alone was 9 days. The median age of women included in the study was 31 years (range 20–51). Characteristics of a cohort of women's had cesarean delivery admitted to the regional women's hospitals of Ukraine are presented in Table II.

ANTIBIOTIC PROPHYLAXIS

In this study 95.9% (2231/2326) of women for whom information was collected were given antimicrobial prophylaxis. In most hospitals (8/11) the first choice of antibiotic agent was cefazoline or cefotaxim. A further four hospitals used ceftriaxone. Of 2326 cesarean delivery participants who underwent chart review, 83.7% were prescribed combination ceftriaxone and metronidazole postpartum. Ceftriaxone and metronidazole was also prescribed for 71.9% participants meeting criteria for SSIs, and 28.1% were prescribed alternative antibiotic regimens. During the study period, there were two maternal deaths in hospitals, none of which were due to infection.

CAUSING PATHOGENS AND ANTIMICROBIAL RESISTANCE

A total of 534 different bacterial strains were isolated from 342 women are with SSIs. Causative microorganisms were recorded for all of the infections. Of these infections 56.1% (192/342) were reported to be polymicrobial. Aerobic gram-negative bacilli make up 50% and 46.3% gram-positive cocci from of all isolates. The most commonly identifed pathogen were *Staphylococcus aureus* (23.5%), *Escherichia coli* (20%). Other pathogens included Coagulase-negative staphylococci (8.1%), *Enterococcus* spp. (7.7%), *Pseudomonas aeruginosa* (7.7%), *Enterobacter* spp. (6.4%), and *Streptococcus* spp. (5.6%) as shown in Table III.

In this study among the atibiotics tested, the ertapenem and piperacillin/tazobactam, and cefotaxim were the most consistently active against Enterobacteriaceae. The overall proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriaceae was 18.3% and of methicillin-resistance in *S. aureus* (MRSA) 13.9%. Against *P. aeruginosa,* the carbapenems (meropenem, ertapenem), and trimethroprim/sulfamethoxazole were the most active agents in infection cases. Vancomycin, teicoplanin, linezolid and fosfomycin, were the most consistently active to the strains of *Enterococcus* spp. Resistance to third-generation cephalosporins was observed in 15.2% *E.coli* and 7.9% *K. pneumoniae* isolates. Carbapenem resistance was identified in 7.3% of *P.aeruginosa* isolates.

DISCUSSION

This study present the first national estimates of the current prevalence rate of SSI (incisional and organ / space) after CSEC in Ukraine using criteria from the CDC/NHSN (USA). The results of SSI surveillance presented in this study constitute a useful source of information on SSIs following CSEC in the participating women hospitals in 2017–2019. A total of 2 326 CSEC operations and 14.7% SSI were identified within 30 days of the operation. Of these, 44.4% were superficial incisional SSI, 28.9% were deep incisional SSI, and 26.6% were organ/space SSI. Of all cases 70.5% of infections were detected post discharge.

The incidence of SSI after CSEC is was 7-10% [11-13]. In European Union Member States for CSEC operations, the percentage of SSIs was 2.2%, with an inter-country range from 0.6% to 7.7% [14]. The percentages of SSIs for CSEC operations in the Ukraine were significantly higher than those reported from EU for 2014–2016 [14] and other countries [11-13]. Perhaps this is due to the use of various methods for definitions of SSIs and the timing of the post-operative surveillance period. In the present study, most of the patients with SSI were diagnosed after discharge from the hospital which is consistent with literature data [15-19].

In the present study, the most frequently identified causing pathogens of SSIs following CSEC were *S. aureus* and *Escherichia coli*, which is consistent with other studies [14, 20, 21]. Other pathogens included Coagulase-negative staphylococci, *Enterococcus* spp., *P. aeruginosa*, *Enterobacter* spp., and *Streptococcus* spp.[21]. The overall proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriaceae was 18.3% and of methicillin-resistance in *S. aureus* (MRSA) 13.9%. Resistance to third-generation cephalosporins was observed in 15.2% *E.coli* and 7.9% *Klebsiella pneumoniae* isolates. Carbapenem resistance was identified in 7.3% of *P.aeruginosa* isolates. According to literature [22-24] and our local antibiotic therapy guidelines, SSIs can be prevented or the rates reduced by including appropriate preoperative antibiotic prophylaxis. However, despite the use of prophylactic antibiotics 14.7% of our patients developed SSI. Perhaps this is due to the high level of antimicrobial resistance of infectious agents that were identified in our study.

STRENGTHS AND LIMITATION

The present study is first step is to quantify this burden for the first time and estimate of the incidence of SSI following CSEC in Ukraine. The strengths of the study lie in the application of CDC/NHSN methodology. The diagnosis of SSI after CSEC based on the CDC / NHSN criteria and where the surveillance period is 30 days. A limitation of the study is that it only include 11 (45.8%) from 24 regional women's hospitals in Ukraine. The results this study may not be representative of other regional women hospitals of Ukraine with different distributions of incidence rate of SSIs after CSEC and antimicrobial resistance of causing pathogens.

CONCLUSIONS

The results of this study revealed high rates of SSI after CSEC and most causing pathogens were associated with resistant to

antibiotic stranis. The most SSI following CSEC is detected after patient's discharge from the hospital. Urgent cesarean operation and improper antibiotic prophylaxis are important risk factors in the development of SSI. This knowledge is essential to develop targeted strategies to surveillance and reduce the incidence of postoperative infections. Further research is required to determine which set of interventions optimize prevetion of SSI after CSEC and improvement in patient outcomes.

REFERENCES

1. WHO Statement on Caesarean Section Rates. https://apps.who.int/iris/bitstream/handle/10665/161442/WHO_RHR_15.02_eng.pdf?sequence=1.
2. Kawakita T., Landy H.J. Surgical site infections after cesarean delivery: epidemiology, prevention and treatment. Matern Health Neonatol Perinatol. 2017; 3: 12. doi: 10.1186/s40748-017-0051-3.].
3. Eurostat. Caesarean births in the EU Member States. https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20191217-1.
4. Li Z., Zeki R., Hilder L., Sullivan E.A. Australia's mothers and babies 2011. Perinatal statistics series no. 28. Cat. no. PER 59. Canberra: AIHW National Perinatal Epidemiology and Statistics Unit. 2013. https://www.aihw.gov.au/getmedia/265f3a72-1ea2-4bff-8a44-b16ca55d00f4/15639.pdf.aspx?inline=true.
5. Betran A.P., Merialdi M., Lauer J.A. et al. Rates of caesarean section: analysis of global, regional and national estimates. Paediatr Perinat Epidemiol .2007;21:98–113. doi:10.1111/j.1365-3016.2007.00786.x.
6. Blumenfeld Y.J., El-Sayed Y.Y., Lyell D.J. et al. Risk factors for prolonged postpartum length of stay following cesarean delivery. Am J Perinatol. 2015;32(9):825–832. doi: 10.1055/s-0034-1543953.
7. Jenks P.J., Laurent M., McQuarry S. et al. Clinical and economic burden of surgical site infection (SSI) and predicted financial consequences of elimination of SSI from an English hospital. J Hosp Infect. 2014;86:24–33. doi:10.1016/j.jhin.2013.09.012.
8. Pierson R.C., Scott N.P., Briscoe K.E., Haas D.M. A review of post-caesarean infectious morbidity: how to prevent and treat. J Obstet Gynaecol. 2018;38(5):591-597. doi: 10.1080/01443615.2017.1394281.
9. Goepfert A.R., Guinn D.A., Andrews W.W. et al. Necrotizing fasciitis after cesarean delivery. Obstet Gynecol. 1997;89(3):409–412. doi: 10.1016/S0029-7844(96)00511-X.
10. Horan T.C., Andrus M., Dudeck M.A. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-332. doi:10.1016/j.ajic.2008.03.002.
11. Centers for Disease Control Surgical Site Infection (SSI) Event. 2018. https://www.cdc.gov/nhsn/pdfs/pscmanual/9pscssicurrent.pdf.
12. Yokoe D.S., Christiansen C.L., Johnson R. et al. Epidemiology of and surveillance for postpartum infections. Emerg Infect Dis 2001;7:837–841.
13. Conner S.N., Verticchio J.C., Tuuli M.G. et al. Maternal obesity and risk of postcesarean wound complications. Am J Perinatol 2014;31:299–304.
14. European Centre for Disease Prevention and Control. Annual Epidemiological Report 2016. Surgical site infections. Stockholm: ECDC. 2016. https://www.ecdc.europa.eu/en/publications-data/surgical-site-infections-annual-epidemiological-report-2016-2014-data.
15. Ward V.P., Charlett A., Fagan J. et al. Enhanced surgical site infection surveillance following caesarean section: experience of a multicentre collaborative post-discharge system. J Hosp Infect. 2008;70(2):166-73. doi: 10.1016/j.jhin.2008.06.002.
16. Barbut F., Carbonne B., Truchot F. et al. Surgical site infections after cesarean section: results of a five-year prospective surveillance. J Gynecol Obstet Biol Reprod (Paris). 2004;33(6 Pt 1):487-96. doi: 10.1016/s0368-2315(04)96561-1.
17. Mitt P., Lang K., Peri A. et al. Surgical-site infections following cesarean section in an Estonian university hospital: postdischarge surveillance and analysis of risk factors. Infect Control Hosp Epidemiol. 2005; 26(5):449-54. doi: 10.1086/502566.
18. Johnson A., Young D., Reilly J. Caesarean section surgical site infection surveillance. J Hosp Infect. 2006;64(1):30-5. doi: 10.1016/j.jhin.2006.03.020.
19. Reilly J., Allardice G., Bruce J. et al. Procedure-specific surgical site infection rates and postdischarge surveillance in Scotland. Infect Control Hosp Epidemiol. 2006;27(12):1318-23. doi: 10.1086/509839.
20. Wloch C., Wilson J., Lamagni T. et al. Risk factors for surgical site infection following caesarean section in England: results from a multicentre cohort study. BJOG. 2012;119(11):1324-33. doi: 10.1111/j.1471-0528.2012.03452.x.
21. Zejnullahu V.A., Isjanovska R., Sejfija Z. et al. Surgical site infections after cesarean sections at the University Clinical Center of Kosovo: rates, microbiological profile and risk factors. BMC Infect Dis. 2019;19(1):752. doi: 10.1186/s12879-019-4383-7.
22. Committee opinion no. 465: antimicrobial prophylaxis for cesarean delivery: timing of administration. Obstet Gynecol. 2010;116(3):791-792. doi: 10.1097/AOG.0b013e3181f68086.
23. Berríos-Torres S.I., Umscheid C.A., Bratzler D.W. et al. Healthcare Infection Control Practices Advisory Committee. Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection, 2017. JAMA Surg. 2017;152(8):784-791. doi:10.1001/jamasurg.2017.0904.
24. Smaill F.M., Grivell R.M. Antibiotic prophylaxis versus no prophylaxis for preventing infection after cesarean section. Cochrane Database Syst Rev. 2014;(10):CD007482. doi: 10.1002/14651858.CD007482.pub3.

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ORCID and contributorship:
Aidyn G. Salmanov: 0000-0002-4673-1154 [A,C,D,E,F]
Alla D. Vitiuk: 0000-0003-0550-1076 [B,C,D,F]
Oleg M. Ishchak: 0000 0002 9634 3635 [B,C,D,F]
Insarova S. Kateryna: 0000-0002-5110-426X [B,C, D,F]
Serhii L. Chyrva: 0000-0002-2848-483 [B, C, D, F]
Marina L. Kuzomenska: 0000-0003-0997-3792 [B, C, D, F]
Oleg V. Golianovsky: 0000-0002-5524-4411 [C, D, F]

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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

A STATISTICAL ANALYSIS OF WOMEN'S REPRODUCTIVE HEALTH CHARACTERISTICS AFTER INEFFECTIVE REATTEMPTS OF USING ART

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Tamara H. Romanenko[1], Anastasiia D. Haiduk[1], Svetlana V. Turbanist[2]
[1]SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION OF THE MINISTRY OF HEALTH OF UKRAINE, KYIV, UKRAINE
[2]ISIDA-IVF MEDICAL CENTER LLC, KYIV, UKRAINE

ABSTRACT

The aim: To carry out a statistical analysis of physical and reproductive health characteristics in women after ineffective reattempts of using assisted reproductive technologies and identify significant medical and social factors contributing to the development of chronic endometritis.

Materials and methods: We carried out a statistical analysis involving 177 outpatient medical records: group 1 includes 127 women aged under 45 with two or more ineffective attempts of in vitro fertilization; group 2 comprised 50 healthy women without reproductive disorders. The complete physical exam included past history, clinical and gynecologic examination, lab techniques and medical imaging according to outpatient medical records obtained during the follow-up care in Isida-IVF Medical Center LLC. Chronic endometritis was recognized by means of histologic examination of endometrium, with the samples obtained by an endometrial pipelle biopsy on day 4-9 of the menstrual cycle.

Results: The prevalence of pelvic inflammatory disease in patients of group 1 comprised 47.2%, endometritis – 41.7%; p1.2 <0.01. The prevalence of acute endometritis among patients of group 1 comprised 6.3%; p1.2 >0.05 and was associated with an infection after abortion. In group 1, sexually transmitted infections were reported in 67.7% of patients, p1.2> 0.05, bacterial vaginosis was registered 1.5 times more often – 28.4%; p1.2 <0.05. In group 1, single induced abortion was reported in 15.0%, while 2 times it took place in 15.7% (p1.2 <0.05). Laparoscopies were previously performed in 59.8% of women, laparotomies – in 62.2%, while in 25.4% of women these interventions were reperformed. Unilateral salpingectomy, due to ectopic pregnancy, was performed in 25.4% of women, bilateral – in 12.7%. Fallopian tube removal, due to sactosalpinx, was performed in 25.4% of women, unilateral salpingectomy took place in 3.9% of cases. The most significant risk factors of chronic endometritis in groups studied are acute postabortal endometritis, RR (relative risk) = 1.439, p = 0.029 and postoperative peritonitis provoked by the complications of appendectomy, RR = 1.430, p = 0.084. We detected none of significant effects of pelvic inflammatory disease on the development of chronic endometritis (RR = 45.132, Confidence interval (CI) (6.151-331.167), p = 0.001). The analysis of reproductive failures, as a risk factor of chronic endometritis in women of group 1, showed a significant relative risk: with the past history of premature birth, RR = 1.44, p = 0.039, in case of reattempts of induced abortions, RR = 1.5, p<0.001, Spontaneous miscarriage RR = 1.572, p = 0.001. A higher relative risk of developing chronic endometritis was observed during pregnancy – RR = 1.597, p = 0.001.

Conclusions: Statistical analysis of women's reproductive health after ineffective reattempts of using ART revealed that the high rate of fetal interventions, low rate of alternative and safe abortion methods are registered in these women, as well as the nonavailability of any system of health measures after reproductive failures. A significant increase in miscarriage at early stages takes place, as well as the absence of a tendency for preterm labor to decrease in the rate. Maintaining women's reproductive health is a considerable medical and social issue, whose resolving depends on joint efforts of governmental and public entities, healthcare agencies and the population responsibility for own health. Designing and adopting the program of preconception care and planning for pregnancy in women after ineffective reattempts of using ART, with chronic endometritis as a primary disease, should be the top priority area of a health care system.

KEY WORDS: assisted reproductive technologies, chronic endometritis, fertilization failure, reproductive health, pregnancy

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INTRODUCTION

The matter of pelvic inflammatory disease these days remains a topical medical and social issue. Pelvic inflammatory disease keeps on being one of the leading causes of reproductive disorders. Along with the high prevalence of cervicitis, vaginitis, salpingo-oophoritis, which occurred, the prevalence of chronic endometritis also rose recently. According to various researchers, the occurrence of chronic endometritis averages out at 14 to 25% of cases. Typically, chronic endometritis is recognized in women with common miscarriage and comprises 6087.5% of cases [1-3].

When using intrauterine contraceptive device the prevalence of chronic endometritis is 6-8% and increases threefold, if such contraception is used longer than five years. The prevalence of chronic endometritis in women, who have repeatedly undergone dilation and curettage for the reason of induced abortion or stillbirth, reaches 12.525% of cases [4, 5].

Chronic endometritis is an inflammatory disease, which is characterized by the impairment of endometrial structure and function, as well as predominantly early pregnancy loss: stillbirth, missed miscarriage, infertility, ineffective attempts of in vitro fertilization and embryo transfer, as well as pregnancy and postpartum complications [2, 3].

Table I. Marital status in women examined (abs. n. %)

Parameter	Groups		Statistical significance of differences
	1 n=127	2 n=50	
First marriage	91 (71.6%)	37 (74%)	p>0.05
Second marriage	9 (7.1%)	13 (26%)	p>0.05
Registered marriage	100 (78.7%)	39 (78%)	p>0.05
Common-law marriage	27 (21.3%)	11 (22%)	p>0.05

Note: $p<0.05$ – statistical significance of differences between groups 1 and 2.

The persistence of infection in endometrium in chronic endometritis, even without prominent disease symptoms, results in the damage to the receptor system in endometrium that may be one of the causes of reproductive disorders in women with intact hormonal ovarian function [4, 6-9].

It is known that risk factors of chronic endometritis are all invasive interventions in the uterine cavity, infection and inflammation as complications after delivery and abortion, intrauterine contraceptive device, vaginitis and cervicitis, cervical stenosis, uterine malformations [10-13]. The primary chronic disease process, involving endometrium in patients having disorders of local immunity [1] with secondary immunodeficiency as a primary disease that occurred due to urogenital infection, cannot be excluded. Despite the adoption of modern technologies for diagnostics and exploration of the brand-new treatment methods, the prevalence of chronic endometritis is not reduced that motivates to explore new approaches to prevention and detection of risk factors.

THE AIM

The aim – to carry out a statistical analysis of physical and reproductive health characteristics in women after ineffective reattempts of using assisted reproductive technologies and identify significant medical and social factors contributing to the development of chronic endometritis.

MATERIALS AND METHODS

We carried out a statistical analysis involving outpatient medical records of 127 women aged under 45 with two or more ineffective attempts of using in vitro fertilization (group1). Inclusion criteria: women's childbearing age – 25-45 years; uterine factor infertility; regular sex life without using contraception for 1 year and more; male fertility. Exclusion criteria: endocrine, tuboperitoneal and/or combined infertility, exacerbation of a chronic physical disorder, subcompensations and decompensations during the last year.

The comparison group (group 2) comprised 50 healthy women without reproductive disorders. To exclude other causes of reproductive disorders, women with severe physical and allergic disorders were excluded, as well as patients with endocrine factor in reproductive disorder, with systemic diseases, congenital thrombophilia, antiphospholipid syndrome, pelvic inflammatory disease in particular; patients, who took immunomodulators less than 6 months ago. The complete physical exam included past history, clinical and gynecologic examination, lab techniques and medical imaging according to outpatient medical records obtained during the follow-up care in Isida-IVF Medical Center LLC. Chronic endometritis was recognized by means of histologic examination of endometrium, with the samples obtained by an endometrial pipelle biopsy on day 4-9 of the menstrual cycle. The techniques for mathematical statistics represented in STATISTICA 6.1 Statsoft Inc were applied in the study, the statistical significance of differences was assessed by the relative risk (RR) value using the following formula: RR = AxD/BxC, where A means the number of patients from the group with chronic endometritis who also represent the feature studied; C – do not have the same characteristic value; B – the number of patients in the control group, who have the feature studied; D – do not have the same feature. To prove that the differences observed between AD and BC are not accidental, the χ^2 criterion was used in the table and for one degree of freedom.

RESULTS

When estimating the age, the mean age of those examined was found to be significantly ($p<0.05$) higher in group 1 and comprised 35.17 ± 0.42, in group 2 this value was 28.30 ± 0.71 years. Group 1 numbered 72 (56.7%) of women of early reproductive age among the patients with ineffective attempts of using ART, 55 (43.3%) of those examined were of late reproductive age. This values came to 47 (94.0%) and 3 (6.0%), respectively, in group 2.

The tendency to later pregnancy planning is registered in patients of group 2. Women of late reproductive age postponed childbearing with an eye on career growth and had a high standard of living. Woman's marital status is a crucial social factor that determines childbearing. The high frequency of marriages registered first-ever was reported in all groups (Table I).

In group I, irrespective of reproductive loss (ectopic pregnancy and missed miscarriage) and infertility, marriages did not break up, while spouses applied for a pre-pregnancy checkup and selection of the method of pregnancy planning. The frequency of unregistered marriages in groups studied did not have any statistically significant differences and came at 27 (21.3%) in group 1, 101 (22.0%) cases in group 2.

Table II. Menstrual cycle characteristics in women examined (M ± m)

Parameters	Groups		Statistical significance of differences
	1 n=127	2 n=50	
Mean age of menarche	13.3±0.1	13.1±0.1	p>0.05
Average duration of menses	5.4±0.1	4.8±0.1	p>0.05
Average duration of the period	29.3±0.2	28.2±0.1	p>0.05

Note: p<0.05 – statistical significance of differences between groups 1 and 2.

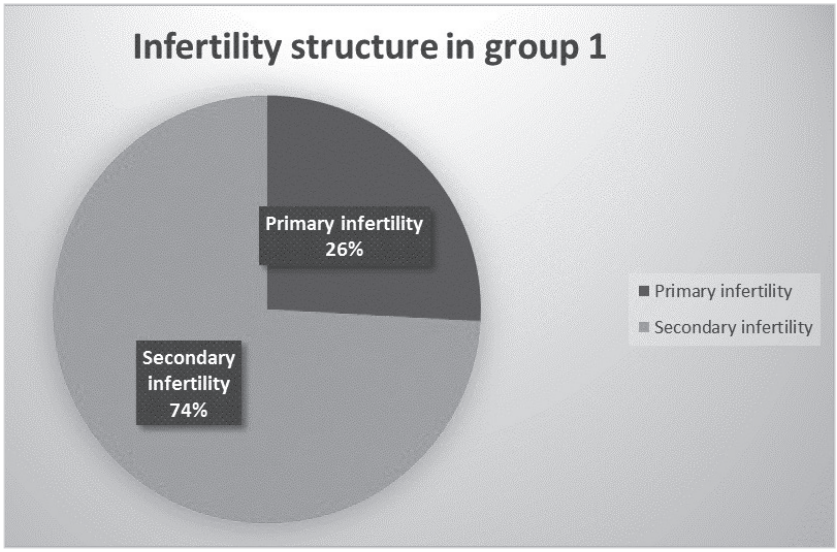

Fig 1. Infertility structure in women of group 1.

When studying women's reproductive health in target groups, we assessed menstrual cycle characteristics, the age of sexual onset, parity. The analysis of menstrual cycle characteristics indicated the age variation at menarche ranging from 11 to 16, depending on the groups it averaged out at 13.3 ± 0.1 and 13.1 ± 0.1 years, respectively (Table II).

Early menarche (11-12 years) was more likely to be registered in patients of group 1 in 27.5% of cases in contrast to 20.0% in group 2 (p<0.05). We did not obtain any statistically significant differences (p>0.05) among patients of groups studied according to the duration of a period and menstrual cycle. The age of menarche corresponds to patients' normal sexual development, while the duration of a period and menstrual cycle complied with characteristics of a normal menstrual cycle. Gonadotropin and steroid evaluation combined with an ultrasound-based monitoring of preovulatory follicle growth indicated the biphasic ovulatory type menstrual cycle in all women from groups studied. Sexual behavior of modern women is characterized by early sexual onset, multiple sex partners. The age of the first pregnancy is 17-18 years, the age of the first childbirth is 25-28 years, over the whole reproductive period woman delivers one-two children. Women and families, willing to have children, reduce in number. Statistically significant differences in the age of sexual onset between patients of group 1 – 17.8 ± 0.2 years and group 2, where this value comprised 18.9 ± 0.3 years (p<0.05) were obtained in the study (TableIII). Every third patient of group 1 (30.7%) had two and more sex partners, 20% of women in control group had 2 sex partners that did not make any statistically significant differences.

The age of the first pregnancy in patients of group 1 (excluding women with primary infertility) was 22.5 ± 0.4 years in such a case, the age of the first delivery was 22.8 ± 0.7 years that indicates the high frequency of delivery resulting from the first pregnancy and the prevalence of secondary infertility. In primary infertility the mean age of sexual onset was 17.3 ± 0.6 years. All women of group 1 complained about reproductive dysfunction. Primary infertility came to 33 (25.9%), secondary – 94 (74.1%) in the structure of reproductive disorders in patients of group 1. Data on the structure of reproductive disorders in women after ineffective attempts of using ART are illustrated in Fig. 1.

The majority of patients from group 1, or 69 (54.4%), applied for a treatment after 5 and more years of infertility. Seventy-eight (61.4%) women applied for the 3rd try of in vitro fertilization, 40 (31.5%) -for the 4th and 9 (7.1%) – for the 5th, and at the same time all patients with primary infertility had failures at trying in vitro fertilization.

Over the last years an increase in the prevalence of reproductive system diseases in women of various ages has become exponential. The structure analysis of gynecological morbidity (Table IV) indicated the high rate of benign diseases and pelvic inflammatory disease.

Typically, patients of group 1 numbered 60 (47.2%) complained about the past history of salpingo-oophoritis, 53 (41.7%) – endometritis, the incidence of acute endometritis among patients of group 1 comprised 8 (6.3%) and

Table III. Features of sexual behavior (M ± m)

Parameter	Groups		Statistical significance of differences
	1 n=127	2 n=50	
Mean age of sexual onset	17.8±0.2	18.9±0.3	p<0.05
Mean age of the first pregnancy	22.5±0.4	25.4±0.6	p<0.05
Mean age at first birth	22.8±0.7	26.2±0.5	p<0.05
Average number of pregnancies per 1 patient	2.4±0.2	1.2±0.1	p<0.05

Note: p<0.05 – statistical significance of differences between groups 1 and 2.

Table IV. Gynecological morbidity in women examined (abs. n. %)

Parameter	Groups		Statistical significance of differences
	1 n=127	2 n=50	
Benign cervical disorders	72 (56.7%)	18 (36%)	p<0.05
Benign ovarian tumors	14 (11%)	2 (4%)	p<0.05
Salpingo-oophoritis	60 (47.2%)	(0%)	p<0.001
Endometritis	53 (41.7%)	(0%)	p<0.001
Endometrial hyperplasia	10 (7.9%)	(0%)	p<0.05
Infertility (I)	33 (25.9%)	(0%)	
Infertility (II)	94 (74.1%)	(0%)	-
BV	36 (28.4%)	9 (18%)	p<0.05
STIs	86 (67.7%)	8 (16%)	p<0.05

Note: p<0.05, p<0.001 – statistical significance of differences between groups 1 and 2.

Table V. The assessment of risk factors of chronic endometritis in pelvic inflammatory disease (abs. n., %, CI, φ)

Parameter	Group 1 n=127		Group 2 n=50		P*	OP (CI 95%)	Strength of the association (φ)
	abs.	%	abs.	%			
Acute postabortal endometritis	8 (6.3%)		(0%)		0.029	1.439 (1.330-1.557)	0.130 p=0.029
Salpingo-oophoritis	43 (33.9%)		(0%)		0.001	45.132 (6.151- 31.167)	0.358 p=0.001
Postoperative peritonitis	4 (3.2%)		(0%)		0.084	1.430 (1.324-1.545)	0.103 p=0.08)

Note: p*– statistical significance of differences between groups 1 and 2.

was associated with postabortal infection. Benign cervical disorders prevailed over pelvic inflammatory disease except for the value in the control group. Predominance of their frequency was reported in patients of group 1 – 72 (56.7%), which is significantly more often (p<0.01) comparing to group 2 – 18 (36.0%). All patients of group 1 took medication (curative treatment with subsequent recovery of normal vaginal flora) and different techniques of radical cervical surgery. Radical techniques for treating cervical ectropion after delivery were used in 8 (16.0%) women of group 2 and they had normal structure and function of cervix till the moment of their inclusion in the study. The occurrence of functional ovarian cysts and ovarian tumors did not make any statistically significant differences in groups, but prevailed slightly in patients of group 1 – 14 (11.0%), which is three times more frequent as compared to group 2 – 2 (4.0%) (p<0.05).

In order to assess risk factors of chronic endometritis in patients of group 1, multiple factor analysis with subsequent determination of relative risk and the strength of the association was carried out. The most significant risk factors of chronic endometritis in groups studied were acute postabortal endometritis, RR = 1.439, p = 0.029 and postoperative peritonitis, resulting from complications of appendectomy- RR = 1.430, p = 0.084. At the same time, we did not detect any significant effect of salpingo-oophoritis on chronic endometritis progression- RR = 45.132, CI (6.151-331.167), p = 0.001 (Table V).

The occurrence of sexually transmitted infections (STIs), dysbiosis, lower genital tract infectious diseases made the

Table VI. Assessment of risk factors of chronic endometritis depending on an infectious agent (abs. n., %, CI, φ)

Parameter	Group 1 n=127		Group 2 n=50		P*	OP (CI 95%)	Strength of the association (φ)
	abs.	%	abs.	%			
Chlamydia infection	19 (15%)		3 (6%)		0.046	2.646 (0.987-7.093)	0.130 p = 0.029
Ureaplasma urealyticum infection	29 (22.8%)		2 (4%)		0.001	5.734 (1.991-6.515)	0.358 p = 0.001
Mycoplasma genitalium (Mgen) infection	20 (15.7%)		4 (8%)		0.824	1.085 (0.527-2.234)	0.103 p = 0.08)

Note: p*– statistical significance of differences between groups 1 and 2.

Table VII. Assessment of risk factors of chronic endometritis that are associated with reproductive history (abs. n., %, CI, φ)

Parameter	Group 1 n=127		Group 2 n=50		P*	OP (CI 95%)	Strength of the association (φ)
	abs.	%	abs.	%			
Normal labor	43 (33.9%)		6 (12%)		0.089	1.735 (0.962-3.130)	0.110 (p=0.065)
Preterm labor	9 (7.1%)		0 (0%)		0.039	1,44 (1.33-1.56)	0.141 (p=0.017)
Induced abortion	47 (37.0%)		0 (0%)		0.01	0.990 (0.591-1.659)	0.450 (p=0.001)
Medical abortion	0 (0.0%)		2(4.0%)		0.001	-	-
First abortion	19 (15.0%)		2(4.0%)		0,163	1.841 (0.773-4.388)	0.083 (p=0.163)
Repeated abortion	20 (15.7%)		0 (0%)		0.001	1.503 (1.376-1.642)	0.242 (p=0.001)
Miscarriage	29 (22.8%)		0 (0%)		0.001	1.572 (1.425-1.735)	0.316 (p=0.001)
Missed miscarriage	37 (29.1%)		0 (0%)		0.001	1.597 (1.443-1.768)	0.338 (p=0.001)

Note: p – statistical significance of differences

past history distinctive in all patients of group 1. In group 1 STIs were registered in 86 (67.7%), BV – 36 (28.4%) (p1.2<0.05), which was fairly more often as opposed to women of group 2: STIs – 8 (16.0%), BV – 9 (18.0%) cases.

We assessed the effect on CE; typically detected in vaginal bacteria biotope (Table VI).

Parameters of confidence intervals and the strength of the association let us suppose that there is no effect of these bacteria on chronic endometritis that proves the numerous researchers' opinion about the overestimation of infectious agents' contribution to pathogenesis of chronic inflammation of endometrium.

The study of reproductive history of women in target groups revealed the high parity of pregnancies among patients in group 1 with secondary infertility. The average number of pregnancies per 1 patient in group 1 came to 1.6 ± 0.02, in group 2 – 0.3 ± 0.01 (p1.2 <0.001). We did not get any statistically significant differences in the number of deliveries in women of group 1, and the relative risk of the effect on chronic endometritis turned out to be low, RR = 1.735, (CI = 0.962-3.130), p = 0.089.

We analyzed the structure of perinatal loss in the past history of women from group I with the history of secondary infertility. In group 1, miscarriage/stillbirth, intrapartum, early neonatal and infant deaths occurred in normal timely birth and comprised 3.2%, 1.1%, 2.1% and 2.1%, respectively. In two cases, miscarriage/stillbirth was recognized as a manifestation of placental insufficiency in case of full-term pregnancy in women, who have previously got abortions. Cases of early neonatal death resulted from intrauterine hypoxia and birth asphyxia. Mortality among infants was due to the effects of birth injuries: one newborn died because of Hypoxic-Ischemic Encephalopathy, one because of sepsis as a complication. Two cases of perinatal death due to congenital malformations took place in group 2.

The analysis of reproductive history associated with invasive interventions in the uterine cavity in the cohort is as

Table VIII. Structure of surgical procedures among women examined (abs. n., %)

Parameter	Groups		Statistical significance of differences
	1 n=127	2 n=50	
Laparoscopy	76 (59.8%)	2 (4%)	p<0.001
Laparotomy	79 (62.2%)	1 (2%)	p<0.001
Salpingectomy	66 (52%)	(0%)	p<0,001
Salpingo-oophorectomy	5 (3.9%)	(0%)	p<0.05
Ovarian surgery	14 (11%)	1 (2%)	p>0.05
Appendectomy	15 (11.8%)	2 (4%)	p<0.05
Peritonitis	5 (3.9%)	(0%)	p<0.05
Cholecystectomy	6 (4.7%)	(0%)	p<0.05
Uterine myomectomy	8 (6.3%)	(0%)	p<0.05

Note: p<0.05, p<0.001 – statistical significance of differences between groups 1 and 2.

Table IX. Assessment of risk factors of chronic endometritis in case pelvic surgery (abs. n., %, CI, φ)

Parameter	Group 1 n=127		Group 2 n=50		P*	OP (CI 95%)	Strength of the association (φ)
	abs.	%	abs.	%			
Salpingectomy	33 (26.0%)		0 (0%)		0.001	1.532 (1.397-1.680)	0.276 (p=0.001)
Salpingectomy due to ectopic pregnancy	22 (17.3%)		0 (0%)		0,001	1.500 (0.962-3.130)	0.110 (p=0.065)
Salpingectomy due to sactosalpinx	11 (8.7%)		0 (0%)		0.005	1.503 (1.374-1.638)	0.238 (p=0.001)

Note: p – statistical significance of differences

follows: the number of induced abortions in women of group 1 turned out to be as follows: single induced abortion was reported in 19 (15%), 2 times it took place in 20 (15.7%), numerous abortions – 10 (7.8%) (p1.2<0.05), miscarriage – 29 cases (22.8%) and missed miscarriage – 37 cases (29.1%).

We comprehensively characterized risk factors of chronic endometritis and reproductive failures by means of statistically significant differences, OR and the strength of the association (Table VII). When studying sexual behavior and early pregnancy failure in target groups we identified risk factors that provoke CE. While identifying risk factors of chronic endometritis we obtained the following results: past history of premature birth, RR = 1.44, p<0.039. The most significant risks were reported in repeated abortions-RR = 1.503, p<0.001; miscarriage – RR = 1.572, p<0.001. Higher relative risk of chronic endometritis was observed in missed miscarriage – RR = 1.597, p<0.001.

Data on the number of surgical procedures in women with different variants of reproductive loss are represented in Table VIII. The frequency of pelvic and abdominal surgery appeared to be reliably higher in group, at the same time laparoscopy was performed in 76 (59.8%), laparotomy – in 79 (62.2%) and in every fifth woman 27.9% of surgical procedures were reperformed, the average number of surgical procedures per 1 patient was 1.2 ± 0.06. The frequency of surgical procedures is 6 times lower in control group, laparotomy prevailed, the average number of surgical procedures per 1 patient was 0.2 ± 0.01.

It is worth mentioning that the frequency of fallopian tube procedures was higher in group 1. Unilateral salpingectomy for the reason of ectopic pregnancy was performed in every fifth patient – 25.4%, bilateral – in every tenth woman (12.7%). Salpingectomy for the reason of sactosalpinx was performed in every fifth woman – 25.4%, unilateral salpingectomy took place in 3.9% of patients. The frequency of ovarian laparoscopic surgery (cystic degeneration was an indication for ovarian wedge resection) came up to 11.0%, 2.0% had resection due to a ruptured corpus luteum cyst in control group. It is noteworthy that laparotomy and laparoscopy were used equally in all groups that indicated the insufficient use of endoscopic technologies when providing gynecological urgent care (Table IX).

Thus, statistical analysis of women's reproductive health after ineffective reattempts of using ART revealed that the high rate of fetal interventions, low rate of alternative and safe abortion methods are registered, as well as the nonavailability of any system of health measures after reproductive failures. A significant increase in miscarriage at early stages takes place, as well as the absence of a tendency for preterm labor to decrease in the rate.

DISCUSSION

To implement the objectives of our study, we assessed women's reproductive health in target groups, analyzed risk factors of reproductive failures and chronic endometritis during the preconception period (stage 1 of the study). Based on the analysis of risk factors for reproductive failures and chronic endometritis during the preconception period (stage 1 of the study) it was identified that the risk of reproductive failures is determined by:

1) Nonavailability of a well-defined system for pregnancy planning and low coverage of the female cohort with modern contraception methods that contribute to high prevalence of benign ovarian tumors and ectopic pregnancy, observed in patients of group 1. Ectopic pregnancy prevailed in group 1: it was reported once in 35 patients (27.5%), twice – in 17 (13.4%), 3 times – in 2 (1.6% (p1.2<0.001)).
2) The high occurrence of salpingo-oophoritis in patients of group 1 – 47.2%, endometritis – 41.7% p1.2<0.01. The frequency of acute endometritis among patients of group 1 comprised 8 (6.3% (p1.2>0.05) and resulted from postabortal infection.
3) The occurrence of Sexually transmitted infections, dysbiosis and lower genital tract infectious diseases, in group 1 STIs were registered in 67.7%, p1.2>0.05, the frequency of BV was 1.5 times higher – 28.4%, p1.2<0.05.
4) The high occurrence of induced abortions, miscarriage, missed miscarriage is a manifestation that burdens reproductive history, was registered in women of group 1: single induced abortion was reported in 15.0%, 2 times it took place in 15.7% (p1.2<0.05). The number of abortions per woman on average was the largest in group I (p <0.05).
5) The high frequency of all pelvic and abdominal surgery techniques, as well as gynecologic surgery was reported in women of group 1. Laparoscopies were previously performed in 59.8%, laparotomies – in 62.2%, at the same time in every fifth woman (25.4%) surgical procedures were reperformed. Unilateral salpingectomy, due to ectopic pregnancy, was performed in every fifth woman – 25.4%, bilateral in every tenth – 12.7%. Fallopian tube removal, due to sactosalpinx, was performed in every fifth woman – 25.4%, unilateral salpingectomy took place in 3.9% of patients.
6) The multiple factor analysis showed that the most significant risk factors of chronic endometritis in target groups are acute postabortal endometritis, RR = 1.439, p = 0.029 and postoperative peritonitis, provoked by the complications of appendectomy – RR = 1.430, p = 0.084.
7) The analysis of reproductive failures, as a risk factor of chronic endometritis in women of group 1 revealed the significant relative risk: with the past history of premature birth, RR = 1.5, p<0.001, spontaneous miscarriage, RR = 1.572, p = 0.001. A higher relative risk of chronic endometritis was observed in missed miscarriage – RR = 1,597, p = 0,001.

Designing and adopting the program of preconception care and planning for pregnancy in women after ineffective reattempts of using ART, with chronic endometritis as a primary disease, should be the top priority area of a health care system.

CONCLUSIONS

Statistical analysis of women's reproductive health after ineffective reattempts of using ART revealed that the high rate of fetal interventions, low rate of alternative and safe abortion methods are registered in these women, as well as the nonavailability of any system of health measures after reproductive failures. A significant increase in miscarriage at early stages takes place, as well as the absence of a tendency for preterm labor to decrease in the rate.

Maintaining women's reproductive health is a considerable medical and social issue, whose resolving depends on joint efforts of governmental and public entities, healthcare agencies and the population responsibility for own health. Designing and adopting the program of preconception care and planning for pregnancy in women after ineffective reattempts of using ART, with chronic endometritis as a primary disease, should be the top priority area of a health care system.

REFERENCES

1. Bochkov V.V., Plehanov A.N., Tsyidenova Ts.B. Hronicheskiy nespetsificheskiy endometrit: epidemiologiya, etiologiya, patogenez, faktoryi riska, diagnostika. [Chronic non-specific endometritis: epidemiology, etiology, pathogenesis, risk factors, diagnostics.] Herald of Buryat State University. 2015; 12: 30 – 38. (in Russian).
2. Kozyireva E.V., Davidyan L.Yu., Kometova V.V. Hronicheskiy endometrit v aspekte besplodiya i nevyinashivaniya beremennosti. [Chronic endometritis in terms of infertility and non-gestation.] Ulyanovsky Medical-Biological Journ. 2017; 2: 56 – 62. doi:10.23648/UMBJ.2017.26.6218. (in Russian).
3. Plyasunova M.P., Hlyibova S.V. Hronicheskiy endometrit kak odna iz aktualnyih problem v sovremennoy ginekologii. [Chronic endometritis as one of the current problems in modern gynecology.] Vyatsky Medical Journal. 2013; 13: 1 – 18. (in Russian).
4. Manuhin I.B., Sementsova N.A., Mitrofanova Yu.Yu., Livshits L.Yu. Hronicheskiy endometrit i nevyinashivanie beremennosti. [Chronic endometritis and lack of pregnancy.] Medical Board. 2018; 7:46-49. doi: 10.21518/2079-701X-2018-7-46-49. (in Russian).
5. Pluzhnikova T.A., Komarov E.K. Znachenie pregravidarnoy podgotovki zhenschin s nevyinashivaniem beremennosti v anamneze. [Importance of pregravitary training of women without pregnancy in anamnesis.] Obstetrics and Women's Diseases Journ. 2015. (in Russian).
6. Akopians A.L., Pisarska M.D., Wang E.T. The Role of Inflammatory Pathways in Implantation Failure:Chronic Endometritis and Hydrosalpinges. Semin. Reprod. Med. 2015; 33 (4): 298–304. doi:10.1055/s-0035-1554916.
7. Kitaya K., Matsubayashi H., Yamaguchi K. et al. Chronic endometritis: potential cause of infertility and obstetric and neonatal complications. Am. J. Reprod. Immunol. 2016; 75 (1): 13–22. doi: 10.1111/aji.12438.
8. McQueen D.B., Bernardi L.A., Stephenson M.D. Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. Fertil. Steril. 2014; 101 (4): 1026–1030. doi: 10.1016/j.fertnstert.2013.12.031.

9. Bouet P.E., El Hachem H., Monceau E. et al. Chronic endometritis in women with recurrent pregnancy loss and recurrent implantation failure: prevalence and role of office hysteroscopy and immunohistochemistry in diagnosis. Fertil. Steril. 2016;105(1):106–110. doi: 10.1016/j.fertnstert.2015.09.025.
10. Lyizikova Yu.A., Zinovkin D.A. Klinicheskaya harakteristika patsientok s besplodiem i immunogistohimicheskaya otsenka ekspressii Foxp3 i CD56 v endometrii. [Clinical characteristics of patients with infertility and immunogistically-chemical evaluation of expression of Foxp3 and CD56 in endometry.] Health and Environment. 2019; 1:35-40. (in Russian).
11. Farquhar C., Marjoribanks J. Assisted reproductive technology: an overview of Cochrane Reviews [Electronic resource]. Cochrane Database of Systematic Reviews. 2018;8. http://www. cochranelibrary.com.
12. Karpeev C.A., Tapilskaya N.I. Sostoyanie mikrobiotyi u patsientok s privyichnyim nevyinashivaniem beremennosti. [Microbiota in patients with a habitual lack of pregnancies.] Obstetrics and Gynecology of Saint Petersburg. 2018; 3(4): 8 – 13. (in Russian).
13. Kamath M.S., Bosteels J., D'Hooghe T.M. et al. Screening hysteroscopy in subfertile women and women undergoing assisted reproduction. Cochrane Database of Systematic Reviews. 2019:4. doi: 10.1002/14651858.CD012856.pub2.

ORCID and contributionship:
Tamara H. Romanenko: 0000-0003-0157-6223 [A,E,F]
Anastasiia D. Haiduk: 0000-0002-7242-2494 [A,B,C,D]
Svitlana V. Turbanist: 0000-0002-8733-1966 [C,D]

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CORRESPONDING AUTHOR
Anastasiia D. Haiduk
Shupyk National Medical Academy of Postgraduate Education of the Ministry of Health of Ukraine
9 Dorohozhytska St., 04112 Kyiv, Ukraine
tel: +380930096329
e-mail: haiduk.anastasiia@gmail.com

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ORIGINAL ARTICLE

INTEGRATIVE MORPHOMETRIC CHARACTERISTIC OF ENDOTHELIAL DYSFUNCTION IN THE CASES OF CHILDREN WITH ESSENTIAL ARTERIAL HYPERTENSION

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Olesya M. Horlenko[1], Vasyl I. Rusyn[1], Viktoriya M. Studenyak[1], Nataliia V. Sochka[1], Fedir V. Horlenko[1], Ivan I. Kopolovets[2,3], Lyubomyra B. Prylypko[1]

[1]STATE HIGHER EDUCATIONAL INSTITUTE "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE

[2]UZHHOROD NATIONAL UNIVERSITY, RESEARCH DEVELOPMENT AND EDUCATIONAL CENTER OF MOLECULAR MICROBIOLOGY AND MUCOSAL IMMUNOLOGY, UZHHOROD, UKRAINE

[3]CLINIC OF VASCULAR SURGERY, EASTERN SLOVAK INSTITUTE OF CARDIOVASCULAR DISEASES AND FACULTY OF MEDICINE, PAVOL JOZEF SAFARIK UNIVERSITY, KOSICE, SLOVAK REPUBLIC

ABSTRACT

The aim: To optimize the treatment of children with Essential Arterial Hypertension (EAH) in assotiation with Endotelial Dysfunction (ED) by studying the clinical and morphofunctional characteristics of the cardiovascular system disorders and correction of endothelial dysfunction with the using of essential phospholipids.

Materials and methods: The study group consisted of 80 children and 30 – a control group. The next stage included the division of 80 children into 2 subgroups. Patients in the first subgroup received basic treatment (angiotensin-converting enzyme inhibitor of the third generation), the second – optimized treatment (basic treatment was with addition of certified drug lecithin). Doses were determined according to the instructions and age for 2 months. In the study were used: ECG, Echocardiography, Ultrasonography, Morphofunctional studies of the endothelium.

Results: There is a dynamic decreasing in the level of left ventricular myocardial mass index (LV MMI), reduction of end-diastolic volume (EDV) and increase in the absolute values of shock volume (SV), ejection fraction(EF) under the influence of optimized treatment due to the inclusion of lecithin in the treatment of children with EAH with ED. The Ve/Va ratio had a tendency to increase. Vasoconstriction of vessels after the reactive hyperemia test was significantly reduced, but the degree of vasodilation varied depending on the method of therapy. The intima-media thickness (IMT) decreased in 1.12 times in the cases of children with an optimized treatment, accompanied by a decreasing of DEC by 2-times. Levels of the aortic stiffness index had a tendency of decreasing (from 0.88 ± 0.02 to 0.71 ± 0.01 and to 0.63 ± 0.01, respectively, by groups and in comparison with the control group – 0.55 ± 0 , 01), which reflects the improvement of hemodynamic parameters. The dynamic parameters obtained in the cases of patients with EAH in association with ED, taking into account the impact of the optimized treatment had positive correction on the total risk of cardiovascular complications, changes in the profile of LV diastolic filling, dysfunction of arterial endothelium.

Conclusions: The inclusion of essential phospholipids in the treatment of children with EAH and ED helps to optimize the profile of LV diastolic filling and exclude vascular endothelial dysfunction and indicate a positive effect of optimized treatment on the overall risk of cardiovascular complications.

KEY WORDS: Essential Arterial Hypertension, Endothelial Dysfunction, children

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INTRODUCTION

Despite progress in the prevention, diagnosis, treatment and control of high blood pressure (BP), there is a further negative trend towards an increasing in the incidence of essential hypertension (EAH) in the pediatric population. Almost every third child with hypertension may develop hypertension disease in the future, which determines the importance of early diagnosis and prevention of hypertension, when the increase in blood pressure is not yet stable. However, clinical and pathogenetic features of EAH in childhood have not been studied enough. This is due to the presence of various pathogenetic mechanisms of EAH development and the peculiarities of its clinical manifestations in the cases of children and insufficient study of the factors through which the main mechanisms of cardiovascular remodeling are realized [1,2].

The endothelium is an active endocrine organ, the largest in the body, diffusely scattered along with the vessels in all tissues. According to the classical definition of histologists, the endothelium is a single-layer layer of specialized cells that line the inside of the entire cardiovascular tree, weighing about 1.8 kg. This is one trillion cells with complex biochemical functions, including systems for the synthesis of proteins and low molecular weight substances, receptors, ion channels [3]. In a broad sense, ED is a condition of vascular endothelium that is accompanied by impaired vasotonic, remodeling, anti-inflammatory and anticoagulant functions. ED is the first link in the pathophysiology

of the cardiovascular continuum, which is based on progressive vascular damage, exacerbated by hypertension. Considering the functions of the endothelium, we can talk about a very complex mechanism of interaction of antagonistic mediators, as well as their regulation of various physiological effects. The spectrum of biologically active substances released by it, changes dramatically, when the function or structure of the endothelium is violated. Under adverse conditions, the endothelium becomes the initiator (or modulator) of many pathological processes in the body. Steady increasing in vascular tone leads to restructuring in the vascular wall [4]. Stages of structural changes are preceded by functional disorders caused by an imbalance in the ratio of vasoconstrictor and vasodilator reactions. Violation of this balance, which is manifested in the development of an imbalance between factors secreted by endothelial cells, is accompanied by the development of endothelial dysfunction.

THE AIM

The aim to optimize the treatment of children with EAH in association with ED by studying the clinical and morphofunctional characteristics of the cardiovascular system disorders and the using of essential phospholipids for the correction of endothelial dysfunction.

MATERIALS AND METHODS

The study group consisted of 110 children. At the first stage, 80 adolescents, aged 15–17 years (average age 16.0 ± 0.11) were examined, among them were 32 boys (40.0 ± 7.75) and 48 girls (60.0 ± 7, 75), in cases of which endothelial dysfunction was identified. The average height of adolescents was 172.0 ± 0.99 cm, which corresponds to 50 centiles, body weight – 46.0 ± 1.01 kg, which corresponds to 10 centiles in children. 2 groups were formed: the first group consisted of 80 children with endothelial dysfunction, the control group included 30 healthy children with identical parameters.

The second stage included the distribution of the first group of children into 2 subgroups. Patients of the first subgroup used basic treatment (angiotensin-converting enzyme inhibitor of the third generation), the second – optimized treatment (basic treatment supplemented with a certified drug, lecithin). Doses were determined according to the instructions and age for 2 months.

ECG, echocardiography, ultrasonography, morphofunctional investigation of the endothelium were used in the study. The diastolic function of the left ventricle was investigated according to the method by L.K. Hattle, B. Angelsen,1985 [5] in pulsed Doppler mode with mapping of the transmitral flow from the apical access of the heart. The assessment of LV diastolic function was performed on the indicators of flow velocities in the phases of peak early diastolic (Ve) and peak late diastolic flow (Va), their ratio (Ve / Va). The aortic stiffness index was defined as the ratio between pulse BP (PBP) and stroke volume (SV).

For endothelial dysfunction identification, a test of reactive hyperemia was performed using a TOSHIBA Aplio 400 (device with a linear sensor with a frequency of 7.5 MHz). The children had to lie on their backs for at least 10 minutes, before the examination. Next, the longitudinal scan measured the diameter of the brachial artery (in the diastole phase) and blood flow velocity (average result for 4 cardiac cycles). Estimation of the diameter of the brachial artery 2 – 5 cm above the elbow flexion. An air cuff was placed on the upper third of the shoulder, creating a pressure that exceeded the systolic blood pressure by 50 mm. rt. art. (5 minutes). Next, the velocity of the indicators was calculated and the diameter of the artery was measured at 30 and 60 s after rheocclusion. The criterion for the presence of endothelial dysfunction is an increase in the diameter of the brachial artery less than 10% [6].

The endothelial dysfunction was characterized by the following parameters:

V max – Maximum systolic (or peak) velocity is the actual maximum linear velocity of blood flow along the axis of the vessel, expressed in mm/s, cm/s or m/s.

V min – Minimum diastolic linear blood flow velocity along the vessel.

V mean – The velocity integral under the curve enveloping the blood flow spectrum in the vessel.

RI (Resistivity Index, index Purcell) – vascular resistance index. RI = (V systolic – V diastolic) /V systolic. Displays state resistance of blood flow distal to the place of measurement.

PI (Pulsatility Index, Gosling index) – pulsation index, indirectly reflecting the state of resistance blood flow. PI = (V systolic – V diastolic)/V mean. This indicator is more sensitive than RI, because in the calculations V mean is used, which responds sooner on the changes in diameter and vascular tone than V systolic. PI, RI must be analysed together because they signify different properties of blood flow in the artery. Using only one of them can cause diagnostic errors.

Endothelial damage was assessed by determining the number of desquamated endothelial cells (DEC) in the blood plasma of patients. The Micros MCX-100 Daffodil microscope was used for the work. The method of determination was carried out as follows. A positive pressure that exceeds systolic blood pressure by 40 – 50 mm. rt. art. is created in the shoulder area, after 4 minutes a decompression is produced, then venous blood collection is carried out, followed by determination of the number of desquamated endothelial cells. Endothelial damage was assessed by determining the number of desquamated endothelial cells (DEC) in the blood plasma of patients with a value greater than 2.77 × 105 in 1 liter of plasma. The method has a high sensitivity and allows to diagnose latent endothelial dysfunction [7]. The Micros MCX-100 Daffodil microscope was used for work.

The results of the work were considered by the commission on bioethics. The study followed the rules of health safety and preserved the rights of the examined children. There were no violations of moral and ethical norms during the research.

Table 1. Date of reactive hyperemia test

Date	Before compression n=80	After compression n = 80
Vmax, cm /s	86,8 ± 1,02	104,1 ± 2,16
Vmin, cm /s	0	1,14 ± 0,06
Vm mean, cm /s	9,48 ± 0,59	8,61 ± 0,63
PI, mm	1,00 ± 0,04	4,5 ± 0,27
Ri,mm	5,71 ± 0,69	0,91 ± 0,07
ΔD, %		8,59
IMT, mm	0,56 ± 0,03	0,56 ± 0,03

Table 2. Indicators of the reactive hyperemia test in the dynamics of treatment in the children with EAH with ED, M ± m

Date	before compression		after compression	
	Basic therapy n=40	Optimized therapy n=40	Basic therapy n=40	Optimized therapy n=40
Vmax (cm /s)	79,12±2,01	86.80±1,02	95,36±3,12	104.1±2,16
Vmin (cm /s)	0,15±0,01	0	1,02±0,31	1.14±0,06
Vm mean (cm /s)	5,57±0,37	5,48±0,59	7,61±0,42	8.61±0,63*
PI (mm)	8,64±1,78	8,40±0,04	5,31±1,73*	4.52±0,27*
RI (mm)	1,00±0,02	1,00±0,69	0,90±0,11	0.91±0,07
ΔD (%)	0	0	8,86	9,69
IMT (mm)	0,56±0,03	0,55±0,02	0,56±0,03	0,50±0,01

Note. * p <0.05 between groups

RESULTS

The results of outpatient measurements revealed significant differences between systolic blood pressure (148.92 ± 0.74 mm Hg versus 109.46 ± 1.32 mm Hg, p <0.001) and diastolic blood pressure (92.90 ± 1.78 mm Hg vs. 64.11 ± 2.97 mm Hg, p <0.001) in the main group and control group. Metabolic changes and increased bioelectrical activity of the left ventricle during ECG examination were also detected. Changes in the morphofunctional characteristics of the left ventricle in the cases of children were insignificant, significant changes were observed between myocardial mass index of the left ventricle (LV MMI), final systolic volume of the left ventricle (LV FSV) compared with children in the control group.

A reactive hyperemia test was performed in the children studied contingent for the endothelial dysfunction detecting. The data is presented in table 1.

The initial diameter of the brachial artery in cases of children of the main groups was significantly larger compared to the control group during the performance of reactive hyperemia test. The increasing in artery diameter (ΔD) was less than 10% in the study group that signifies the presence of endothelial dysfunction [6]. There was also an increasing of *Intima-media* thickness (IMT) (0.56 ± 0.03 mm), which, cannot exceed 0.41 ± 0.004 mm according to the authors data, in identical cases of healthy children from the same age group [8].

Below the ultrasonograms in the studied contingent are presented (Fig. 1,2)

Consider a set of dynamics indicators which characterize the endothelial dysfunction (table 2).

The initial diameter of the brachial artery in main groups children was significantly larger compared to the control group. The artery diameter increasing after reactive hyperemia test in these groups was less than 10%, which is a criterion of the endothelial dysfunction presence. Evaluation of the study of echostructural properties of the carotid arteries showed that in the cases of children of the main groups there is a significant increasing in the thickness of the intima-media complex. The artery diameter after reactive hyperemia test had tendency to increasing, but only in the cases of children receiving optimized treatment and approached to the reference limit (from 8.59% to 9.69%). At the beginning of the study, there was an increase in intima-media thickness (IMT) (0.56 ± 0.03 mm), the level of which in the dynamics of treatment decreased more significantly in the group with the optimized method of treatment (from 0.56 ± 0.03 mm to 0 , 55 ± 0.02 mm and from 0.56 ± 0.03 mm to 0.50 ± 0.01 mm and in relation to the data of the control group 0.44 ± 0.02 mm). The vascular pulsation index (VI) was significantly reduced (by 2 times) in the group of children with optimized treatment, but did not reach the reference upper limit. In the control group of children after the compression test a significant maximum increasing of vessel diameter (up to 11.61%) was revealed, which corresponds to the satisfactory vascular wall elastic characteristics and the endothelial dysfunction absence.

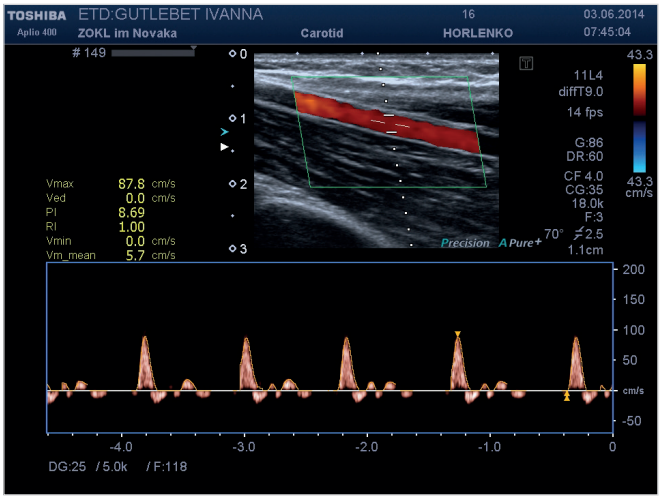

Fig. 1. The state of vascular blood flow before compression

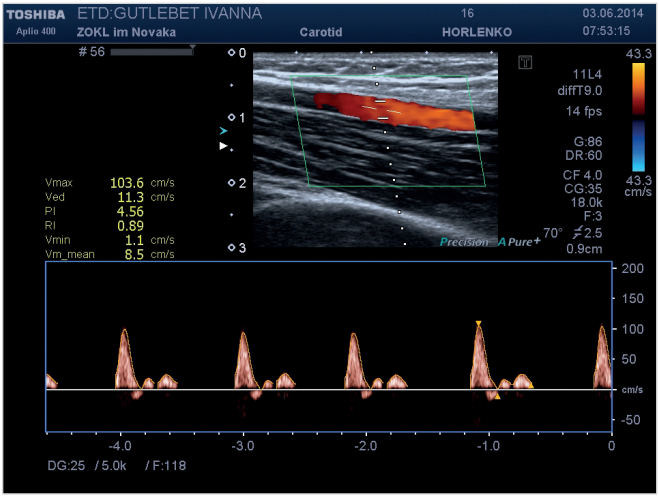

Fig. 2. The state of vascular blood flow after compression

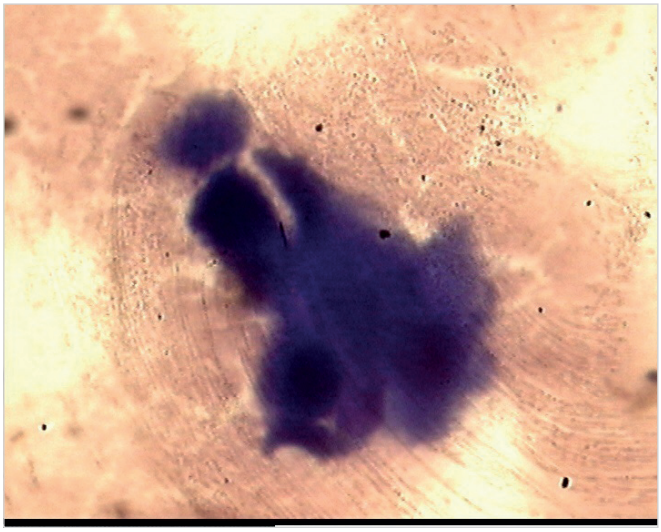

Fig. 3. Indicators of the reactive hyperemia test in the dynamics of treatment in the children with EAH with ED, M ± m

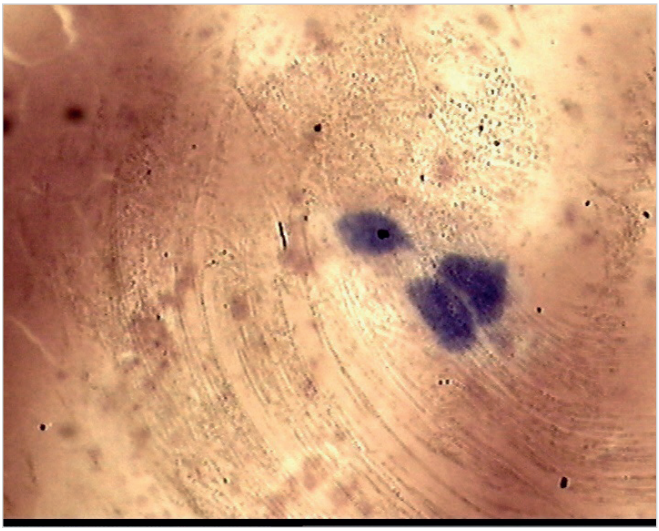

Fig. 4. Complexes of endothelial cells after treatment without signs of polymorphism, the nuclear-cytoplasmic ratio is preserved, fine-grained chromatin. The nucleus is located eccentrically.

Thus, according to our data, hypertension in the cases of children was accompanied by a violation of the elastic properties of the vascular wall.

We performed a dynamic morphometric studies to determine the number of desquamated en dotheliocytes (DEC) in the studied contingent (fig.3,4).

The DEC index was in the range of 3 – 5 cells x 10^5 in 1 liter of blood (4.46 ± 0.27 desquamated endothelial cells) according to our data. After treatment, 2-3 cells x 10^5 in 1 liter of blood (2.28 ± 0.31 desquamated endothelial cells) were observed in the field of view, which indicates a positive dynamics of this indicator. The number of desquamated endothelial cells was 3.56 ± 0.31 x 105 in 1 liter of blood in the cases of children using basic therapy. In the control group of children, the process of desquamation of the endothelium is moderate and can be interpreted as a normal physiological process of purification of the intima from damaged cells.

A dynamic echocardiographic study was also performed in the examined pediatric contingent depending on the treatment method. There is a slight increase in the amplitude of peak E, respectively, in groups and a decrease in the height of peak A in the cases of children of both groups, according to the indicators of transmitral blood flow. The E/A ratio had a tendency to increasing of the levels (from 1.37 ± 0.18 to 1.60 ± 0.11 and up to 1.80 ± 0.23, respectively, by groups). LV IVRT data tend to decrease (from 109.32 ± 9.41 ms to 104.44 ± 8.23 ms and from 109.63 ± 8.39 ms to 79.22 ± 4.41 ms had and in comparison with data from the control group – 72.18 ± 3.41 ms). That is, the IVRT rate remains prolonged, but in the children group of with optimized treatment it is close to the reference.

Changes in echocardiography parameters: decrease in the peak velocity of the E wave; increasing the peak velocity of wave A; reducing the ratio Ve / Va; prolongation of the isovolumic relaxation phase; the increasing in DT time can be regarded as the initial manifestations of diastolic dysfunction in the examined children. Echocardiography data of study indicators in the examined pediatric contingent

are accorded to the characteristics of the model of diastolic dysfunction (DD) of delayed relaxation. Disturbances in the morphofunctional characteristics of the left ventricle were accompanied by changes in the structure of other cavities of the heart, as well as indicators of the central and peripheral hemodynamics. Confirmation of myocardial remodeling processes in adolescents with EAH can be accompanied by change in such indicators, as LV MMI and relative thickness of the posterior wall of the left ventricle (LV RTPW). These indicators in the studied contingent were higher than in the control, according to our data, which are consistent with the data of many authors.

The aortic stiffness index levels also tended to decrease (from 0.88 ± 0.02 to 0.71 ± 0.01 and to 0.63 ± 0.01, respectively, by groups and compared with the control group – 0.55 ± 0.01), which reflects the improvement of hemodynamic parameters. There was an increasing in the time of isovolumic relaxation of IVRT and a decreasing in the rate of early diastolic filling, which are signs of diastolic dysfunction, according to our data.

LV MMI decreased in the dynamics after the treatment and was 75.88 ± 2.43 g / m2 and 73.09 ± 2.18 g / m2, respectively, by groups, which allows us to make conclusions about the reverse trend of left ventricular hypertrophy (LVH) in patients with EAH. This was expressed in a decreasing of end-diastolic volume (EDV) (from 108.13 ± 2.01 ml to 98.97 ± 3.4 ml in the group with optimized treatment and without changes in the group receiving basic treatment) and absolute values increasing of SV (from 65.02 ± 2.73 ml to 70.38 ± 1.78 ml and from 65.02 ± 2.73 ml to 80.10 ± 2.18 ml, respectively, by groups), EF – from 65.94 ± 1.88% to 69.32 ± 1.61% and from 66.27 ± 1.95% to 75.00 ± 1.38%, respectively, by groups). This increasing is characteristic for myocardial hyperkinesia. The vasoconstriction was significantly reduced after the reactive hyperemia test in the cases of patients who have received optimized treatment with lecithin. The obtained data on changes in the profile of LV diastolic filling and arterial endothelial function disorder indicate a positive effect on the total cardiovascular complications risk in the cases of children with EAG in association with ED who have received optimized treatment.

DISCUSSION

Studies have shown that the problem of endothelial dysfunction has proven clinical significance and requires in-depth study in the cases of children, in recent years, because the detection of this condition and timely elimination of its consequences will prevent more serious diseases in adults.

According to the American Society of Echocardiography, there are four reasons for the IMT of the main arteries: age, professional sports, subclinical atherosclerosis and vasculitis [9]. Our study contingent excludes other risk factors for the development of EAG in association with endothelial dysfunction, in addition to the picture of subclinical atherosclerosis with initial vascular damage. In the control group of children, a significant maximum increase in vessel diameter (up to 11.61%) was revealed during the compression test, which corresponds to satisfactory elastic characteristics of the vascular wall and the absence of endothelial dysfunction. Thus, Hypertension in the cases of children was accompanied by a violation of the elastic properties of the vascular wall. However, the normal response of the endothelium of the brachial artery was not fully restored. Although, the degree of vasodilation varied depending on the method of therapy. Our correlation analysis did not reveal a connection between the ability of the brachial artery to dilate, the level of blood pressure, heart rate, EDV, EF and indicators of the phase-volume structure of diastole. LVH also did not correlate with Doppler indices and vasodilating activity of the brachial artery. The indicative fact is that endothelial disorders of dependent relaxation and diastolic LV dysfunction are common, on the base of regression of LVH. We can assume that these processes in the cases of patients with EAG exist side by side and independently of each other. But it is possible, that to identify the dynamic effects of endothelial dysfunction on the persistence of phase-volume characteristics of transmitral blood flow and diastolic dysfunction are need for longer-term observation and examination of patients

According to our data, there was an increase in the time of isovolumic relaxation of IVRT and a decrease in the rate of early diastolic filling, which are signs of diastolic dysfunction. There are three models of DD. The first model of DD is called delayed relaxation, or abnormal relaxation, which is hemodynamically represented by a decrease in the volume of blood entering the ventricle in the early filling phase and an increasing in the contribution of the atria. The mitral valve leaflets open later and the duration of the isovolumic relaxation phase increases, as a result of the decreasing in the rate of drop of intraventricular pressure. Slowing of ventricular relaxation leads to an increasing of the volume of blood in the atrium before the onset of systole, which causes an increasing in the force of atrial contraction [10]. Diastolic dysfunction in the studied contingent is a pathogenetic factor that contributes to the development of clinical manifestations in the cases of children without systolic dysfunction (SV – from 65.02 ± 2.73 ml to 70.38 ± 1.78 ml and from 65.02 ± 2, 73 ml to 73.10 ± 2.18 ml, respectively, by groups and in comparison with the control group (76.01 ± 1.98 ml) with normal EF values. Thus, the obtained data about changes in the profile of diastolic filling of the left ventricle and dysfunction of arterial endothelium due to treatment of patients with EAG in association with ED indicate a positive effect of optimized treatment on the total risk of cardiovascular complications.

CONCLUSIONS

1. The study and the obtained dynamic indicators in the cases of patients with EAH in association with ED, taking into account the developed optimized treatment influence on changes in the profile of LV diastolic filling, arterial endothelial dysfunction confirm the possibility

of positive correction on the total risk of the appearance and development of cardiovascular complications.
2. The blood vessels Vasoconstriction after the reactive hyperemia test was significantly reduced, but the degree of vasodilation varied depending on the method of therapy. The intima-media thickness (IMT) decreased by 1.12 times in the cases of children with an optimized treatment, accompanied decreasing of DEC (by 2 time).
3. There is a dynamic decreasing in the level of left ventricular myocardial mass index (LV MMI), reduction of end-diastolic volume (EDV) and increase in the absolute values of shock volume (SV), EF. The Ve / Va ratio had a tendency to increasing due to the inclusion in treatment of lecithin in the cases of children with EAH with ED
4. Levels of the aortic stiffness index had a tendency to decrease (from 0.88 ± 0.02 to 0.71 ± 0.01 and to 0.63 ± 0.01, respectively, by groups and in comparison with the control group – 0.55 ± 0, 01), which reflects the improvement of hemodynamic parameters.

REFERENCES

1. Biletskyi S.V., Boiko V.V., Petrynych O.A., Kazantseva T.V. Endotelialna dysfunktsiia ta arterialna hipertenziia (ohliad literatury) [Endothelial dysfunction and hypertension (literature review)]. Klinichna ta eksperymentalna patolohiia. 2017;1(59):160-163. (In Ukrainian). https://doi.org/10.24061/1727-4338.XVI.1.59.2017.36.
2. Kvashnina L.V., Ihnatova T.B. Profilaktyka porushen endotelialnoi funktsii u ditei u period perekhodu vid zdorovia do syndromu vehetatyvnoi dysfunktsii [Prevention of endothelial dysfunction in children during the transition from health to autonomic dysfunction syndrome]. Sovremennaia pedyatryia. 2016;5(77):16-24. (In Ukrainian). https://doi.org/10.15574/SP.2016.77.16
3. Kashkalda D.A. 2020 Osoblyvosti zmin stres-rehuliuiuchykh system u pidlitkiv iz sertsevo-sudynnoiu patolohiieiu pry endotelialnoi dysfunktsii [Features of changes in the stress-regulating system in adolescents with cardiovascular pathology with endothelial dysfunction]. Suchasna pediatriia. Ukraina. 2020;5(109):8-14. (In Ukrainian). doi 10.15574/SP.2020.109.8.
4. Gardner A.W., Parker D.E., Montgomery P. et al. Gender and Racial Differences in Endothelial Oxidative Stress and Inflammation in Patients With Symptomatic Peripheral Artery Disease. J Vasc Surg. 2015;61(5):1249-1257. https://doi.org/10.1016/j.jvs.2014.02.045;
5. Hattle L.K., Angelsen B. Doppler ultrasound in cardiology: physical principles and clinical application. Philadelphia; 1985, p.780-890.
6. Haliutina O.Yu. Funktsiia endoteliiu u khvorykh na revmatoidnyi artryt, yii zviazok z metabolichnym syndromom ta perebihom zakhvoriuvannia [Endothelial function in patients with rheumatoid arthritis, its relationship with the metabolic syndrome and the course of the disease]. Bukovynskyi medychnyi visnyk. 2017;1(81):39-43. (In Ukrainian).
7. Hryhorova I.A., Heletka O.O., Stepanenko O.Iu. Kharkivskyi natsionalnyi medychnyi universytet patentovlasnyk. Sposib vyznachennia tsyrkuliuiuchykh deskvamovanykh endoteliotsytiv krovi [Method for determining circulating desquamated blood endothelial cells]. Patent Ukr № 66974. 2012. (In Ukrainian).
8. Marushko Yu.V., Marushko T.V., Artemenko Ye.O. et al. Kardiolohiia dytiachoho viku: navchalnyi posibnyk [Cardiology of a child's life: tutorial]. Kyiv-Khmelnytskyi: pryvatna drukarnia FShch-P Storozhuk O.V.; 2018, p.528. (In Ukrainian).
9. Arrebola-Moreno A.L., Laclaustra M., Carlos Kaski J. Noninvasive Assessment of Endothelian Function in Clinical Practice. Revista Española de Cardiología. 2012;65(1):80-90. https://doi.org /10.1016/j.rec.2011.10.004.
10. Bojchenko A.D., Senatorova A.V., Gonchar' M.A., Kondratova I.Ju. Tipy diastolicheskoj disfunkcii zheludochkov serdca u novorozhdennyh v rannij neonatal'nyj period [Types of diastolic dysfunction of the ventricles of the heart in newborns in the early neonatal period]. Mezhdunarodnyj zhurnal pediatrii, akusherstva i ginekologii. 2014;5(3):10-17. (In Russian).

ORCID and contibutionship:

Olesya M. Horlenko: 0000-0002-2210-5503 [A,D]
Vasyl I. Rusyn: 0000-0001-5688-9951 [A]
Viktoriya M. Studenyak: 0000-0003-3935-0537 [C]
Nataliia V. Sochka: 0000-0002-3973-2976 [B]
Fedir V. Horlenko: 0000-0002-0496-2069 [B]
Ivan I. Kopolovets: 0000-0002-2721-6114 [E]
Lyubomyra B. Prylypko: 0000-0002-4131-5450 [F]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olesya M. Horlenko
Uzhhorod National University
1 squ. Narodna, 88000 Uzhhorod, Ukraine
tel: +380505269658
e- mail: ohorlenko@gmail.com

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ORIGINAL ARTICLE

HEMODYNAMIC ANDCYTOLOGIC CHANGESINPROSTATE GLANDCAUSED BY CHRONIC HEPATITIS

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Volodymyr B. Grytsuliak[1], Mariana M. Vasylechko[1], Oksana I., Kocherzhat[1], Oksana I. Hotiur[2]
[1]V. STEFANYK PRECARPATHIAN NATIONAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE
[2]IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

The aim: Is to determine the features of hemodynamics and the character of structural and functional changes in the prostate gland in middle-aged infertile males in the conditions of chronic hepatitis.

Materials and methods: We studied the features of hemodynamics and structural and functional state of the prostate gland in middle-aged males who had suffered from chronic hepatitis with the help of ultrasound scanning, Color-Doppler sonography, and histology, morphology, and statistics.

Results: It was detected that in 3-5 years after chronic viral hepatitis in middle-aged males (the first period), the volume of the prostate gland increases to (27.1±1.2) cm3, and its mass to (28.0±1.7) g compared to the control. Blood flow rates in the prostate gland: arterial blood flow velocity, blood flow diastolic velocity, volumetric blood flow are significantly lower than the control ones. Glandular zone surface decreases to 53% and connective tissue increases to 43%. Epithelium in the secretory area of the gland is compressed.

Conclusions: 1.According to the data of ultrasound diagnostics and Color-Doppler sonography, the volume of the prostate gland increases to (27.1±1.8) cm^3 and its mass to (28.0±1.7) g at chronic viral hepatitis, compared to the control (21.3±1.5) cm3 and (24.7±1.3) g ($p<0.05$). Atrophic changes in the prostate gland are manifested with the decreased height of the columnar epithelium of the gland and overgrowth of connective tissue in the stroma of the organ. 2.Angioechometric indices of arterial blood circulation in central and peripheral zone of the prostate decreases to (7.26±0.46) cm/sec and (7.80±0.32) cm/sec respectively compared to the control (18.30±3.10) cm/sec and (17.70±2.90) cm/sec. Venous blood flow rates decrease to (2.45±0.21) cm/sec and (2.60±0.16) cm/sec compared to (5.54±0.8) cm/sec and (5.36±0.03) cm/sec ($p<0.05$).

KEY WORDS: prostate gland, hemodynamics, histostructure, chronic hepatitis

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INTRODUCTION

As it is known, the prostate gland serves several important functions in the male reproductive system. In particular, its glandular parenchyma produces mucous secretion which dilutes sperm and improves its motility. It also secretes prostaglandins into the blood what stimulates the testicular hormonal activity of and spermatogenesis [1, 2, 3, 4, 5]. Acute and chronic prostatitis and prostate adenoma are widely described in the literature [6, 7, 8], but the state of hemodynamics and structural and functional changes in it at chronic hepatitis remain studied too little that determines the relevance of this study.

THE AIM

The aim of the study is to determine the features of hemodynamics and the character of structural and functional changes in the prostate gland of middle-aged infertile males with chronic hepatitis.

MATERIALS AND METHODS

The ultrasound scanning and the Color-Doppler sonography of the prostate gland in 12 middle-aged infertile males (22-35-year-old), diagnosed with chronic hepatitis in anamnesis, were performed in a clinical and diagnostic center on the *SIEMENS SONOLINE G60S* ultrasound system (*Siemens AG*, Germany).

We determined the volume of the prostate gland in cm3, its mass using the formula $m=V\times1.05$, where V was the volume of the gland, 1.5 was the coefficient. In the mode of color flow mapping, as well as in pulsed-wave Doppler, we evaluated the character of vascular pattern, vessel diameter. The quantitative evaluation was done according to vascular plexus density, the number of vessels VES/cm3, and the vessel diameter (VD). Hemodynamics qualitative indicators in the prostate gland were: 1) peak arterial flow rate (cm/sec), 2) blood flow diastolic velocity, DV (cm/sec), 3) linear velocity of blood flow (cm/sec), 4) perfusion index, PI (RU), 5) resistive index, RI (RU), 6) volumetric blood flow (L/min). In the archive histological micropreparations of 7 prostate biopsies at this pathology collected in the Department of Urology of CNE "Ivano-Frankivsk Regional Clinical Hospital", we detected relative surfaces of glandular and muscular-elastic components, as well as the height of glandular epithelial cells.

Statistical analysis of the received results was done with the help of the *Statistica 6* software.

Table I. The echometric indicators of the prostate gland in middle-aged infertile males after hepatitis

Type of the test	Prostate parameters (M±m; *p<0.05)				
	Width	Thickness	Length	Volume	Mass
Control	75.5±2.0 (mm)	24.5±1.7 (mm)	22.6±1.6 (mm)	21.3±1.5 (cm^3)	24.7±1.3 (g)
Chronic hepatitis	41.2±1.5*	26.5±1.4	27.3±1.2*	27.1±1.8*	28.0±1.7*

Table II. The blood flow indicators in the prostate gland in middle-aged infertile males after chronic viral hepatitis (M±m), (n=19)

Indicators, measurement units	Central zone	Peripheral zone
Peak arterial flow rate, cm/sec	7.26±0.46	7.80±0.32
Blood flow diastolic velocity, cm/sec	2.45±0.21	2.60±0.16
Linear velocity of blood flow, cm/sec	5.28±0.23	5.40±0.22
Perfusion index, RU	5.42±0.23	5.46±0.18
IP, RU	1.18±0.05	1.20±0.05
Resistive index, cm	0.06±0.01	0.05±0.01
Vascular plexus density, VES/cm^3	0.75±0.43	0.69±0.16
Volumetric blood flow, L/min	0.03±0.001	0.01±0.002

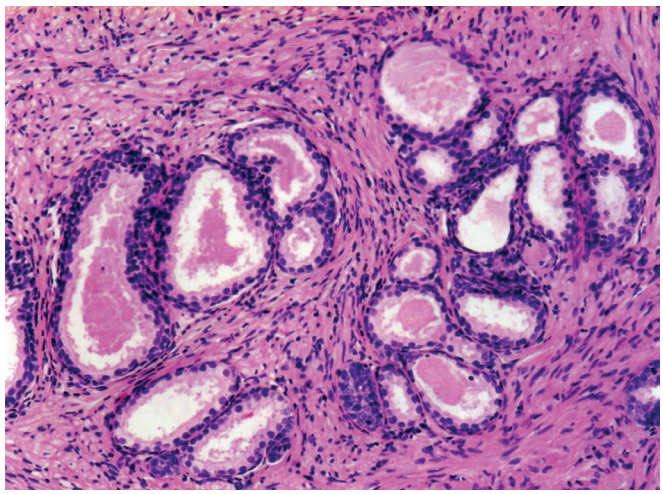

Fig. 1. Expressed overgrowing of connective tissue around prostate particles in a 35-year-old male with chronic hepatitis. Colored with hematoxylin and eosin.

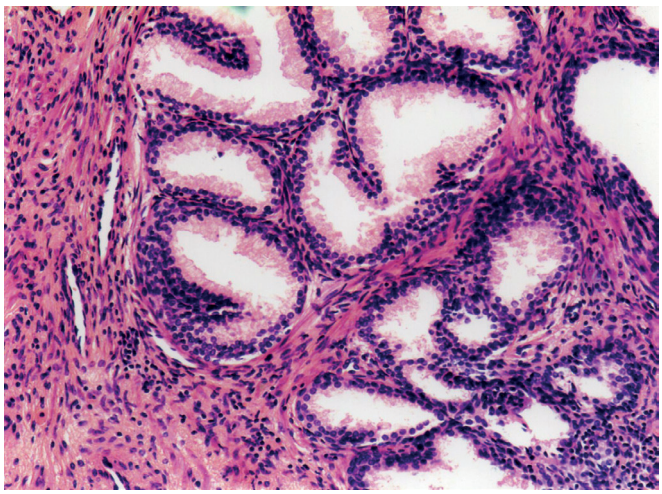

Fig. 2. Terminal secretory zones of the prostate gland in a 32-year-old male are lined with cylindrical epithelium (control). Colored with hematoxylin and eosin.

The ethical committee of V. Stefanyk Precarpathian National University did not detect any ethics violations during the study (Record No2 from 20.10.2020).

RESULTS AND DISCUSSION

According to received data, the volume of the prostate gland increases to (27.1±1.2) cm3, and its mass to (28.0±1.7) g compared to the control (Table I) in middle-aged infertile males with chronic hepatitis in anamnesis.

Blood flow indicators in the prostate gland show under these circumstances that peripheral parenchyma characterizes by lower vascularization compared to the one in the central zone. The vascular pattern is inhomogeneous, disorganized, with a significant decrease of peak velocities and volumetric blood flow due to stagnation (Table II).

In histological micropreparations of the prostate biopsies, the surface ratio of glandular parenchyma to elastic stroma is 52% to 98% compared to the control 70% to 30%. It has been detected an increased number of particles that undergo atrophic processes with decreasing of the height of glandular epithelium (fig. 1) transforming to cubical or squamous ones. There is overgrowing of connective tissue present.

The middle-aged males of the control group have secretory zones of tubular-alveolar particles in the prostate gland. The epithelium of the terminal zones is cubical or cylindrical. In the part of secretory zones, there is an amorphous exprimate. In the prostate stroma, there is loose and smooth muscle tissue (fig. 2).

The used methods of ultrasound scanning and the Color-Doppler sonography of the prostate gland, color flow mapping, and laboratory tests of ejaculate allowed reaching the set goal.

Received results on structural and functional changes in the prostate gland are original because in the processed literature similar data were not found.

There is an established fact of the regulatory influence of testicular hormones on the general state of the human body [1]. The decrease of its endocrine activity is considered one of the main causes which accelerate aging processes in the body. But the spermatogenic function of the testes is more important, its disorders cause male infertility.

The data about the blood supply of testicles and prostate gland are presented in many works [5, 7, 8] but the features of the influence of hepatitis on the blood supply of male reproductive organs in males of reproductive age remain incomplete. This especially concerns the prostate gland, the character of changes in hemomicrocirculatory bed and tortuous spermatic cords, testicle, epididymis at chronic viral hepatitis of the liver.

The authors found out that the ratio between the total lumen of arterial vessels and the volume of the prostate gland change with age [6, 7]; our data show the influence of viral hepatitis by the increase of the volume of the prostate gland to (27.1±1.8) cm3 and its mass to (28.0±1.7) g compared to (21.3±1.5) cm3 and (24.7±1.3) g in the control group ($p<0.05$).

According to the data about disorders in hemodynamics presented by us and other researchers [2, 3, 4], an increase of destructive changes in the wall of the blood vessels of different caliber and ultrastructural rebuild of blood-testis barrier, are ones of the causes in the reduction of germinal epithelium cell layers in tortuous spermatic cords.

Prospects for further studies lie in the study of the influence of chronic hepatitis on the structural and functional state of a testicle and ejaculate.

CONCLUSIONS

1. According to the data of ultrasound diagnostics and Color-Doppler sonography, the volume of the prostate gland increases to (27.1±1.8) cm3 and its mass to (28.0±1.7) g at chronic viral hepatitis, compared to the control (21.3±1.5) cm3 and (24.7±1.3) g ($p<0.05$). Atrophic changes in the prostate gland are manifested with the decreased height of the columnar epithelium of the gland and overgrowth of connective tissue in the stroma of the organ.
2. Angioechometric indices of arterial blood circulation in central and peripheral zone of the prostate decreases to (7.26±0.46) cm/sec and (7.80±0.32) cm/sec respectively compared to the control (18.30±3.10) cm/sec and (17.70±2.90) cm/sec. Venous blood flow rates decrease to (2.45±0.21) cm/sec and (2.60±0.16) cm/sec compared to (5.54±0.8) cm/sec and (5.36±0.03) cm/sec ($p<0.05$).

REFERENCES

1. Schill W. B., Comhaire F. N., Hargreave T. B. Andrology for the Clinical. Springer–Verlag Berlin Heidelberg. 2011, 800 p.
2. Grytsuliak B.V., Dolunko N.P., Popadinec O.H. et al. Kharakter morfofunktsionalnykh zmin v peredmikhuroviy zalozi, zumovlenykh etanolovoyu intoksykatsiyeyu [The nature of morphofunctional changes in the prostate gland, caused by ethanol intoxication]. Bulletin of problems biology and medicine. 2016;2(129):70-73. (in Ukrainian).
3. Gerashchenko S. B., Grytsuliak B. V., Grytsuliak V. B. et al. Osoblyvosti strukturnykh zmin u peredmikhuroviy zalozi v umovakh subtotalnoyi tsyrkuliatornoyi hipoksiyi i stymuliatsiyi krovotoku [Pecularties of structural changes in prostate gland in conditions of subtotal hypoxy and blood flow stimulation]. World of Medicine and Biology. 2011;4:26-28. (in Ukrainian).
4. Grytsuliak B. V., Grytsuliak V. B., Dolynko N. P. et al. Strukturno-funktsionalni zminy v peredmikhuroviy zalozi cholovikiv zriloho viku pislia perenesenoho epidydymoorkhitu [Structural and functional changes in the prostate gland of mature men after epididymo-orchitis]. Bulletin of problems biology and medicine. 2020;2(156):256-259. (in Ukrainian).
5. Naumov N. P., Sheplov P. A., Polozov V. V. The role of antioxidants in prevention of male infertility. Andrology and Genital Surgery. 2019;20(1):13-21.
6. Lytvynets Y. A., Khallo O. Y. Strukturno-funktsionalni osoblyvosti krovonosnoho rusla i parenkhimy peredmikhurovoyi zalozy pry khronichnomu prostatyti [The structural and functional pecularties of the blood channel and the parenchyma of the prostate gland in case of chronical prostatitis]. Clinical Anatomy and Operative Surgery. 2011;2(10):25-28. (in Ukrainian).
7. Glina S., Glina P. Pathogenic mechanisms linking benign prostatic hyperplasia, lower urinary tract symptoms and erectile dysfunction. TherAdv Urol. 2013;5(4):211–218. doi: 10.1177/1756287213488236.
8. Lesin A. I., Lytvynets Y. A., Seyeniv Y. M. Kombinovana konservatyvna terapiya khvorykh iz symptomamy nyzhnikh sechovykh shliakhiv ta yikh uskladnenniamy [Combined conservative therapy of the patients with symptoms of lower urinary tracts and their complications]. Health of Man. 2009;3:180-183. (in Ukrainian).

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ORCID and contributionship:
Oksana I. Hotiur: 0000-0001-9687-0112 [A,B,E,F]
Volodymyr B. Grytsuliak: 0000-0001-5719-748X [B,F]
Mariana M. Vasylechko: 0000-0001-9640-47X [B,D]
Oksana I. Kocherzhat: 0000-0003-4568-586X [B,C]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Oksana I. Hotiur
Ivano-Frankivsk National Medical University
17 Fedkovych St., 76008 Ivano-Frankivsk, Ukraine
tel: +380996616005
e-mail: Oksanahotiur@gmail.com

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ORIGINAL ARTICLE

THE EFFECT OF COMPREHENSIVE TREATMENT OF PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE IN COMBINATION WITH PREDIABETES ON THE LIPID PROFILE

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Vitalina V. Ivachevska
STATE HIGHER EDUCATIONAL ESTABLISHMENT «UZHHOROD NATIONAL UNIVERSITY», UZHHOROD, UKRAINE

ABSTRACT

The aim: To evaluate the efficiency of the proposed therapy, which included recommendations for nutrition, physical activity and treatment with rosuvastatin, omega-3 PUFA and ursodeoxycholic acid, on the indicators of the lipid profile in patients with NAFLD and prediabetes.

Materials and methods: 78 patients with impaired glucose tolerance were examined. According to the inclusion and exclusion criteria, 55 patients with prediabetes and concomitant NAFLD were included in the study. All patients underwent a comprehensive clinical examination, which included anthropometric data collection, objective examination, and venous blood sampling for laboratory tests.

Results: The data obtained after 12 months of proposed treatment revealed a statistically significant improvement of indicators lipid profile in patients with prediabetes and NAFLD. Moreover, no significant difference between mean values of HDLC, LDLC, TG and atherogenic coefficient of almost healthy individuals and the corresponding indicators of treated patients detected.

Conclusions: therapy which included recommendations for nutrition, physical activity and treatment with rosuvastatin, omega-3 PUFA and ursodeoxycholic acid significantly improved lipid metabolism in patients with prediabetes and NAFLD.

KEY WORDS: non-alcoholic fatty liver disease, prediabetes, lipid metabolism, rosuvastatin, omega-3 PUFA and ursodeoxycholic acid

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered to be the most common cause of chronic liver disease worldwide [1,2]. In the general population the prevalence of NAFLD is about 20-30% [3], however, the prevalence is much higher in high-risk groups, which include obese people with type 2 diabetes mellitus, metabolic syndrome [4].

Data from epidemiological studies [3,5-8] confirm the tendency of increased incidence of NAFLD in patients with insulin resistance, which occurs in patients with obesity, type 2 diabetes and metabolic syndrome. While in the general population NAFLD is found in 30% [3], it reaches an impressive frequency among patients with grade I and grade II-III obesity – 75% [5,9,10] and 90% [7,8], respectively. NAFLD is found in 90% of patients with diabetes and hyperlipidemia [11].

NAFLD is very serious condition as it can lead to the development of non-alcoholic steatohepatitis (NASH) and unfortunately to cirrhosis, without the any signs and symptoms or changes of the liver enzymes. It was confirmed that patients with prediabetes and NAFLD are at higher risk of development of diabetes mellitus type 2 comparing with prediabetes patients without NAFLD [12].

It is common to see an increase of free fatty acids (FFA), low-density lipoprotein (LDL), and triglycerides (TG) as as a manifestation of dyslipidemia in patients with NAFLD. Such abnormalities of lipid metabolism cause inflammation, lipotoxicity, oxidative stress and as the result aggravate liver damage [13].

Interestingly, the prevalence of NAFLD among patients with prediabetes, as well as the features of metabolic disorders in this category of patients compared with patients with type 2 diabetes remain poorly studied. The question of the choice of treatment approach in patients with NAFLD and prediabetes also remains debatable.

THE AIM

To evaluate the efficiency of the proposed therapy, which included recommendations for nutrition, physical activity and treatment with rosuvastatin, omega-3 PUFA and ursodeoxycholic acid, on the indicators of the lipid profile in patients with NAFLD and prediabetes.

MATERIALS AND METHODS

On the basis of the therapeutic department of the State Enterprise "District clinical hospital of Uzhgorod station" State Territorial Branch Union (STBU) "Lviv railway" examined 78 patients with impaired glucose tolerance. Ac-

cording to the inclusion and exclusion criteria, 55 patients with prediabetes and concomitant NAFLD were included in the study. The exclusion criteria were: age over 74 years; documented coronary heart disease; connective tissue disease; oncological diseases; the presence of complete left bundle branch block according to the ECG; type I and II diabetes mellitus; cirrhosis; previous viral hepatitis; toxic (alcohol – consumption of more than 40 g of ethanol /d), drugs (use of hepatotoxic drugs), severe metabolic liver disease; Wilson-Konovalov disease; autoimmune hepatitis; abrupt weight loss, parenteral nutrition for 2 weeks or more; chronic diseases of the gastrointestinal tract, accompanied by impaired absorption function (malabsorption syndrome); worm infestation; refusal of the patient to participate in this study.

Patients were divided into 2 groups, which were comparable in age, sex and stage of NAFLD. The division of patients into groups was performed in random order, by random numbers.

Because the initial examination of patients with prediabetes and NAFLD revealed that they belonged to the category of people at very high cardiovascular risk, all patients were prescribed rosuvastatin at a dose of 10 mg /d. Thus, patients of group Ia (n = 28) as hypolipidemic therapy received rosuvastatin 10 mg / d in combination with omega-3 PUFA at a dose of 1000 mg /d and ursodeoxycholic acid at a dose of 10 mg/kg/d.

Patients of group Ib (n = 27) were a comparison group and, except for rosuvastatin, did not take drugs. Patients in both groups followed dietary recommendations and performed 30-minute daily walks. For comparison, a control group of almost healthy individuals was used (n = 30). Evaluation of treatment efficacy was performed 6 and 12 months after the start of therapy.

All patients underwent a comprehensive clinical examination, which included anthropometric data collection, objective examination, and venous blood sampling for laboratory tests.

The method of ultrasound diagnosis of the liver was used to diagnose NAFLD, taking into account that ultrasound can detect an increase in liver echogenicity and confirm the diagnosis of NAFLD in conditions of fatty infiltration of the liver more than 33%. Criteria for the presence of steatosis were considered to be a diffuse homogeneous increase in liver echogenicity ("white liver") with greater echogenicity than the right kidney (hepatorenal index) and dorsal attenuation of the ultrasound signal.

Statistical processing of the obtained results was performed on a personal computer using the software packages "Microsoft Office Excel 2003" and "Statsoft Statistica 8.0". The discrepancy was considered probable if the probability value was equal to or greater than 95% ($p < 0.05$).

RESULTS

During the study period among patients of group Ia who received complex treatment, there was a clear tendency to improve lipid metabolism (table I): the average value of total cholesterol significantly decreased after 12 months by 37.96% compared to baseline ($p < 0.01$) and 26.65% compared with data after 6 months ($p = 0.03$). In addition, the average value of total cholesterol after 12 months in patients of group Ia was significantly lower than in almost healthy individuals ($p = 0.02$). The mean value of high-density lipoprotein (HDL) in patients of group Ia after 12 months significantly increased by 42.19% compared to baseline ($p < 0.01$) and by 23.81% compared with the mean value after 6 months of treatment ($p = 0.01$). The mean value of LDL levels significantly decreased after 12 months of treatment by 10.90% compared with the mean value before treatment ($p < 0.01$).

Moreover, a significant difference between the mean values of HDL and LDL levels of almost healthy individuals and the corresponding indicators of patients of group Ia after 12 months was not detected ($p = 0.19$ and $p = 0.49$, respectively). There was a significant decrease in the mean value of very low-density lipoprotein (VLDL) levels both after 12 months compared to baseline (by 12.21%, $p < 0.01$) and after 12 months compared with the average value after 6 months of treatment (by 9.45%, p). = 0.03). The mean value of triglyceride level significantly decreased after 12 months of treatment by 40.23% ($p < 0.01$) compared to baseline and by 30.74% compared with 6 months ($p = 0.03$).

Thus, due to the general tendency to improve lipid metabolism, patients in this group showed improvement of the atherogenic coefficient, namely: at the beginning of the study, this coefficient indicated a moderate risk of atherosclerosis, after 6 months of treatment, the atherogenic coefficient decreased significantly by 12.67% ($p < 0.01$), thus approaching normal.

After 12 months of treatment, the atherogenic coefficient decreased by 32.78% ($p < 0.01$) and was within normal levels, as evidenced by the lack of significant changes between its average value after 12 months and the average value of this ratio in healthy individuals (group III) ($p = 0.07$).

Among patients of group Ib, statistically significant changes in the mean values of some indicators of lipid metabolism were found (table II). Thus, the average value of total cholesterol significantly decreased after 12 months of treatment by 17.60% compared to baseline ($p < 0.01$) and by 14.46% compared with the average value of this indicator after 6 months of treatment ($p < 0, 01$). Moreover, the average value of total cholesterol after 12 months in patients of group Ib did not differ significantly from the average value of this indicator in almost healthy individuals ($p = 0.16$). No significant change was in the mean value of HDLC in patients of group Ib ($p > 0.05$), while the average value of this indicator in patients of group Ib was significantly lower than in almost healthy individuals ($p = 0.01$). No significant change was in the mean values of LDL and VLDL ($p > 0.05$), as well as no significant difference was between the mean values of these indicators in patients of group Ib and persons in the control group ($p = 0.11$ and $p = 0.88$, respectively). During the study period, there was a significant decrease in the average value of triglycerides in patients of group Ib after 12 months by 15.31% ($p = 0.04$),

Table I. Dynamics of lipid profile of patients of group Ia during the treatment (M ± m)

Indicators	Group of comparisons				Reliability indicator			
	Group Ia (n=28)			Control group (n=30)	p0-6	p0-12	p6-12	p12-control group
	Baseline	After 6 months	After 12 months					
Total Cholesterol	5,19±1,06	4,39±2,08	3,22±1,72	4,03±0,66	0,08	<0,01*	0,03*	0,02*
HDLC	1,28±0,36	1,47±0,46	1,82±0,42	2,27±1,76	0,09	<0,01*	0,01*	0,19
LDLC	3,12±0,50	3,03±0,58	2,78±0,43	2,71±0,34	0,54	0,01*	0,07	0,49
VLDLC	1,31±0,18	1,27±0,24	1,15±0,16	0,83±0,13	0,48	<0,01*	0,03*	<0,01*
TG	3,43±1,38	2,96±1,47	2,05±1,54	1,84±0,26	0,22	<0,01*	0,03*	0,47
Atherogenic coefficient	3,63±0,29	3,14±0,58	2,44±0,74	2,15±0,43	<0,01*	<0,01*	<0,01*	0,07

n- number of patients; p0-6 - the significance of the difference of the indicators of patients in the relevant group before treatment and after 6 months; p0-12 - the significance of the difference of the indicators of patients in the relevant group before treatment and after 12 months; p6-12 - the significance of the difference of the indicators of patients in the relevant group after 6 and 12 months of treatment; p12-III - the significance of the difference between the patients of the corresponding group after 12 months of treatment and patients of control group; * - statistically significant difference when comparing indicators in the dynamics.

Table II. Dynamics of lipid profile of patients of group Ib during the treatment (M ± m)

Indicators	Group of comparisons				Reliability indicator			
	Group Ib (n=27)			Control group (n=30)	p0-6	p0-12	p6-12	p12-control group
	Baseline	After 6 month	After 12 month					
Total Cholesterol	5,17±1,07	4,98±0,71	4,26±0,54	4,03±0,66	0,46	<0,01*	<0,01*	0,16
HDLC	1,17±0,34	1,21±0,47	1,23±0,39	2,27±1,76	0,72	0,55	0,87	0,01*
LDLC	3,11±0,51	3,08±1,24	3,02±0,97	2,71±0,34	0,91	0,67	0,84	0,11
VLDLC	0,77±0,14	0,78±0,61	0,81±0,72	0,83±0,13	0,94	0,78	0,87	0,88
TG	4,18±1,44	3,96±1,21	3,54±0,61	1,84±0,26	0,55	0,04*	0,11	<0,01*
Atherogenic coefficient	2,72±0,34	2,60±0,49	2,39±0,37	2,15±0,43	0,30	<0,01*	0,08	0,03*

n- number of patients; p0-6 - the significance of the difference of the indicators of patients in the relevant group before treatment and after 6 months; p0-12 - the significance of the difference of the indicators of patients in the relevant group before treatment and after 12 months; p6-12 - the significance of the difference of the indicators of patients in the relevant group after 6 and 12 months of treatment; p12-III - the significance of the difference between the patients of the corresponding group after 12 months of treatment and patients of control group; * - statistically significant difference when comparing indicators in the dynamics.

but the average value of this indicator after 12 months was significantly higher than in almost healthy individuals (p <0, 01). Like the mean triglyceride level, the mean value of atherogenic coefficient significantly decreased after 12 months compared to baseline by 12.13% (p <0.01), but remained significantly higher than in almost healthy individuals (p = 0, 03).

DISCUSSION

The results of many studies demonstrate the effectiveness of ursodeoxycholic acid, omega-3 PUFA and rosuvastatin in patients with NAFLD but independently of each other. For instance, scientists Anita Pathil, Gerhard Liebisch and co- authors, as well as E. B. Avalueva, E. I. Tkachenko and co-authors in their studies demonstrated a restoring altered lipid profiles of in patients with NAFLD which were treated with ursodeoxycholic acid [14,15]. Schohraya Spahis, Fernando Alvarez and co- authors showed beneficial effect of omega-3 fatty acids on liver steatosis and related metabolic abnormalities in obese children with NAFLD [16]. S. Antonopoulos and co-researchers in their work confirmed the positive effect of rosuvastatin on the course of non-alcoholic fatty liver disease [17]. At the same time, Srinivasan Dasarathy and others did not show a positive effect from the use of omega 3 fatty acids in diabetic patients with NAFLD [18]. In our study, we demonstrated for the first time the effectiveness of comprehensive treatment of NAFLD in prediabetes patients and its positive influence on lipid abnormalities.

CONCLUSIONS

Thus, it can be concluded that standard nutrition and physical activity recommendations, as well as treatment with rosuvastatin in patients with prediabetes was significantly effective, but did not provide complete correction of lipid metabolism.

At the same time, proposed treatment, which included recommendations for nutrition, physical activity and treatment with rosuvastatin, omega-3 PUFA and ursodeoxycholic acid, demonstrated significant reduction in the mean values of total cholesterol and VLDL. As well as the lack of a statistically significant difference in the mean values of other indicators of lipid profile in patients with prediabetes and NAFLD compared with almost healthy individuals proves the efficacy of the proposed treatment in patients with prediabetes and NAFLD.

REFERENCES

1. Li B., Zhang Ch., Yu-Tao Zh.. Nonalcoholic Fatty Liver Disease Cirrhosis: A Review of Its Epidemiology, Risk Factors, Clinical Presentation, Diagnosis, Management, and Prognosis. Canadian Journal of Gastroenterology and Hepatology. 2018. doi.org/10.1155 .
2. Wong V.W., Chu W.C., Wong G.L. et al. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. Gut. 2012;61:409-415.
3. Pais R., Barritt A.S. 4th., Calmus Y. et al. NAFLD and liver transplantation: Current burden and expected challenges. J Hepatol. 2016;65(6):1245-1257.doi: 10.1016.
4. Portillo-Sanchez P., Bril F., Maximos M. et al. High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. J Clin Endocrinol Metab. 2015;100(6):2231-2238. doi:10.1210/jc.2015-1966.
5. Akbar D.H., Kawther A.H. Nonalcoholic fatty liver disease in Saudi type 2 diabetic subjects attending a medical outpatient clinic: Prevalence and general characteristics. Diabetes Care. 2003; (26):3351-3352.
6. Bellentani S., Saccoccio G., Masutti F. et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. Ann. Intern. Med. 2000; 132:112-117.
7. Dixon J.B., Bhathal P.S., O'Brien P.E. Nonalcoholic fatty liver disease: Predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese.Gastroenterology.2001;121:91-100.
8. Garcia-Monzon C., Martin-Perez E., Iacono O.L. et al. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. Hepatol. 2000; 33:716-724.
9. de Costa G. A. et al . Non-alcoholic fatty liver disease (NAFLD) in different populations: A clinical and epidemiological study – sample of São José do Rio Preto. Rev. Assoc. Med. Bras. São Paulo. 2016;62(3):218-226.
10. Hazlehurst J.M., Woods C., Marjot T. et al. Non-alcoholic fatty liver disease and diabetes. Metabolism.2016;65(8):1096-1108. doi: 10.1016 /j.metabol. 2016. 01. 001.
11. Targher G., Day C.P., Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N. Engl. J. Med. 2010;363:1341-1350.
12. Nishi T., Babazono A., Maeda T. et al. Evaluation of the fatty liver index as a predictor for the development of diabetes among insurance beneficiaries with prediabetes. J Diabetes Investig. 2014;6:309–316.
13. Pei K., Gui T., Kan D. et al. An Overview of Lipid Metabolism and Nonalcoholic Fatty Liver Disease. Biomed Res Int. 2020;4020249.
14. Pathil A., Liebisch G., Okun J.G. et al. Ursodeoxycholyl Lysophosphatidylethanolamide modifies aberrant lipid profiles in NAFLD. Eur J Clin Invest. 2015;45(9):925-31. doi: 10.1111/eci.12486.
15. Avalueva E.B., Tkachenko E.I., Skazyvaeva E.V. et al. Efficiency of ursodeoxycholic acid therapy in non-alcoholic fatty liver disease associated with metabolic syndrome. Eksp Klin Gastroenterol. 2013;(11):26-30.
16. Spahis S., Alvarez F., Ahmed N. et al. Non-alcoholic fatty liver disease severity and metabolic complications in obese children: impact of omega-3 fatty acids. J Nutr Biochem. 2018;58:28-36. doi: 10.1016/j. jnutbio.
17. Antonopoulos S., Mikros S., Mylonopoulou M. et al. Rosuvastatin as a novel treatment of nonalcoholic fatty liver disease in hyperlipidemic patients. Atherosclerosis. 2006; (84): 233-234.
18. Dasarathy S., Dasarathy J., Khiyami A. et al. Double-blind randomized placebo-controlled clinical trial of omega 3 fatty acids for the treatment of diabetic patients with nonalcoholic steatohepatitis. J Clin Gastroenterol. 2015;49(2):137-144.

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ORCID and contributionship:
Vitalina V. Ivachevska: 0000-0002-2036-3568 [A,B,C,D,E,F]

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CORRESPONDING AUTHOR
Vitalina V. Ivachevska
Uzhhgorod National University
148 Sobranetska st., 88015 Uzhhgorod, Ukraine
tel: +380506766803
e-mail: vitalina.ivachevska@uzhnu.edu.ua

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ORIGINAL ARTICLE

EPIDEMIOLOGICAL FEATURES OF SARS2 COVID-19 OUTBREAK DURING 2020 AMONG RT-PCR CONFIRMED CASES IN ZAKARPATTYA

DOI: 10.36740/WLek202104128

Stepan O. Karabinyosh, Galina M. Koval
STATE HIGHER EDUCATION ESTABLISHMENT UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: The overall aim of this project was to provide a comprehensive description of epidemiological and demographic features of SARS2 COVID19 outbreak among patients in Zakarpattya oblast during 2020 to define most vulnerable and susceptible groups of population.

Materials and methods: We conducted a retrospective observational descriptive study of the 33 190 cases that tested RT-PCR positive for SARS 2 COVI19 in Zakarpattya oblast, studied their comorbidities and concomitant diseases, as well as their clinical and functional outcomes.

Results: Among studied cases: females – 20 069 (60.47±0.27%) and males – 13 121 (39.53±0.27%).

Geographical distribution showed that: 10 230 (30.8%) of cases were registered in district capital Uzhhorod and surrounding area, 3 646 (11%) were registered in Mukachevo district, Tyachivsky district 3 235 cases (9.7%), Vynohradisky district 2 627 (7.9%), other districts had less than 4 %. Most patients were within the age group 31 and 70 with a mean age of 47.76±17.57 years.

Conclusions: the emergence of current outbreak of SARS-CoV-2 (COVID-19) pandemic is a reminder of how new pathogens can mutate, emerge and rapidly spread through large amount of human population, and cause severe burden to public health and healthcare system is general.

KEY WORDS: SARS-CoV-2, COVID-19, Emergence, Distribution, Zakarpattya

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INTRODUCTION

Coronavirus infection (COVID19) – is a severe acute respiratory disease caused by novel coronavirus which belongs to *Coronaviridae* family. They are positive-sense RNA viruses and may infect a large amount of hosts. Gene sequencing and phylogenetic study shows that SARS-CoV2 which caused the world pandemic outbreak belongs to the order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae, genus Betacoronavirus. It has the genera Alphacoronavirus and Betacoronavirus originate from bats [1].

The first cases of novel COVID-19 disease were registered in December of 2019 in Wuhan, China [2]. It occurred through zoonotic transmission and was related to seafood market infection. However, later it has been reported that the disease was rapidly spreading from human-to-human and that the actual numbers were underestimated [3].

Covid19 is a highly contagious respiratory infectious disease usually manifested with mild symptoms (around 80%) or even asymptomatic (1-2%). The median age of COVID-19 cases ranges from 49 to 57 years [4].

Transcarpathian region (Zakarpattya oblast) is a western administrative division of Ukraine, which borders with four European countries such as Poland, Slovakia, Hungary and Romania. The number of Ukrainian immigrants in neighbouring European countries accounts a large population [5].

Such location of the district and its geopolitical issues related to immigration plays a crucial role in diseases transmission and serves as a gate to infectious diseases to and from European Union [6].

According to the reports of Ministry of Healthcare of Ukraine the first case in Ukraine has been registered on March 3, 2020 in Chernivtsi. On March 13, the first fatal case of coronavirus infection was recorded. According to the System of monitoring of the distribution of COVID infection "SOTA" which is powered by National Security and Defense Council of Ukraine and other open source data provided by Ministry of Healthcare of Ukraine, as of February 14, 2021, there were 1 271 143 infected people in Ukraine, of whom 24 330 died (1.9±0.01%), 1 114 301 recovered (87.66±0.03%), 132 512 currently ill (10.4±0.03%) and 1 387 290 cases suspected with disease (result anticipated).

The outbreak made a tremendous impact on quality of life in general, as well as it is being a huge burden on healthcare system and economy of the country. The most cases were registered in the capital of the country 111 471 cases, followed by Odessa oblast 71 125 cases, Kharkiv oblast 69 377 cases, and Lviv oblast 61 268 cases. Which can be explained by the fact that these are the biggest transportation hubs of the country. It has been observed that there is a significant correlation between amount of transportation and cumulative cases of COVID19 [7].

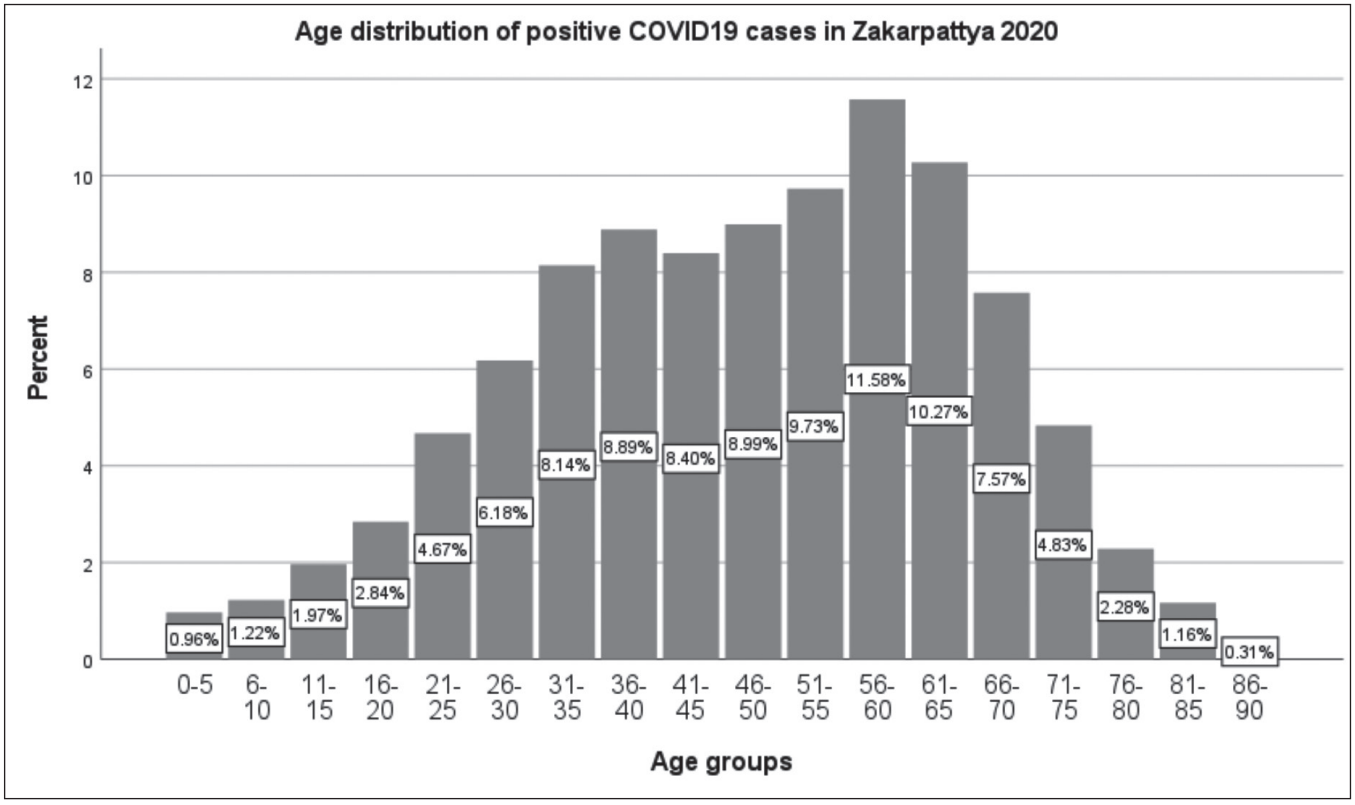

Fig. 1. Age groups of patients who were tested RT-PCR positive for COVID19 in Zakarpattya oblast in 2020.

THE AIM

The overall aim of this project was to provide a comprehensive description of epidemiological and demographic features of SARS2 COVID19 outbreak among patients in Zakarpattya oblast during 2020 to define most vulnerable and susceptible groups of population.

MATERIALS AND METHODS

We conducted a retrospective observational descriptive study of the 33 190 cases that tested RT-PCR positive for SARS 2 COVI19 in Zakarpattya oblast and registered in Electronic Integrated Information System for Infectious Disease Surveillance (ELISS), studied their comorbidities and concomitant diseases, as well as their clinical and functional outcomes.

RESULTS

Among studied cases: females – 20 069 (60.47±0.27%) and males – 13 121 (39.53±0.27%). Almost 8% (2 588 cases) were healthcare workers.

Geographical distribution (Table. I) showed that: 10 230 (30.8%) of cases were registered in district capital Uzhhorod and surrounding area, 3 646 (11%) were registered in Mukachevo district, Tyachivsky district 3 235 cases (9.7%), Vynohradisky district 2 627 (7.9%), other districts had less than 4 %.

Most commonly patients who were tested positive were within the age group between 31 and 70 with a mean age of 47.76 (95% CI 47.57 – 47.95) (Fig. 1). The oldest patient was 97 years old who successfully recovered from the disease. However, we studied age distribution divided into groups, and discovered that the biggest number falls between 56 – 60 years – 11.58% (3 839 cases), followed by age group 61 – 65 – 10.27% (3 406 cases), and 51 – 55 years – 9.73% (3 226).

There was also some certain seasonality observed with the highest number of cases per day in November (09 Nov 2020 – 490 cases) (Fig. 2) with mean of case reporting 105±5.21 (95% CI 94.77 – 115.28).

With regards to hospital care – 7 363 (22.2%) cases were hospitalized to inpatient facility, and 25 724 (77.5%) were treated in outpatient clinic. Among analysed cases 9 557 (28.8%) had concomitant diseases: Cardiovascular diseases: 6 727 (20.3%), Diabetes: 1 223 (3.7%), 2% had chronic lung diseases, 1.7% of cases had chronic neurological or neuromascular diseases or oncology, and approximately 1% reported chronic liver or kidney diseases (Table II). However, 3 363 (10%) were unable to respond whether they have any chronic conditions or diseases. Though, only 10% required oxygen insufflation and 3.8% had Acute Respiratory Distress Syndrome and were admitted to intensive care facility.

Out of analysed cases (Table III), with obtained information on the outcome (n=4016): 726 cases (2.2%) resulted in fatality, 27 874 cases (84%) recovered, and 573 (12.1%) cases were remaining ill at the moment of data analysis.

Table I. Distribution of COVID19 among districts of Zakarpattya oblast during 2020

Name	N	%
1. Beregivsky	1729	5.2%
2. Velykobereznyansky	1230	3.7%
3. Vynogradivsky	2627	7.9%
4. Volovetsky	660	2.0%
5. Irshavsky	1600	4.8%
6. Mizhgirsky	1090	3.3%
7. Mukachivsky	3646	11.0%
8. Perechynsky	1713	5.2%
9. Rahivsky	2253	6.8%
10. Svalyasky	1215	3.7%
11. Tyachisky	3235	9.7%
12. Uzhgorodsky	10230	30.8%
13. Hustsky	1657	5.0%
14. Chopsky	305	0.9%

DISCUSSION

The distribution of the disease shows prevalence of cases in highly populated area like district capital. However, this also may be explained by lack of accessibility to healthcare facilities in remote mountain areas.

We also suggest that such distribution could also be due to the fact, that the districts with highest number of cases are located near border crossing points and therefore have large number of incoming people from abroad. The least cases are on the border with two of Ukrainian oblast Lviv and Ivano-Frankivsk (Fig. 3), even though Lvivska oblast accounted to one of the biggest numbers of positive cases in the country in 2020 – 61 268 cases (5.81±0.02%) [6, 8, 9].

Among the analyzed cases majority 77.5% were treated at outpatient clinic and only 22.2% required specialized care at inpatient facility. According to the local Standard of Covid19 diagnostic and treatment, the most common indications for hospitalization: moderate and severe course of the diseases, ARDS, severe intoxication, sepsis, etc. However, studies show that only 5 – 10 % require specialized care. This can be explained, with the fact that majority of hospitalized cases were admitted without actual need and were due to miscommunication regarding actual state of the patient. [10]

The study we performed shows that cardiovascular diseases and hypertension are the most common comorbidities among COVID-19 cases that we analysed which have also been described in the literature. Comorbidities reported by patients show that majority were not previously informed about their underlying diseases. This can be explained with the fact that medical reform in Ukraine is still being implemented and some patients do not have their permanent general practitioner or family doctor yet, and therefore unaware of any chronic diseases. [11]

The study was limited to data available in the reports submitted to ELISS. Further studies should follow up with analysis of patients who were hospitalized, because it would give a greater perspective and understanding of clinical manifestation, treatment outcome and prognosis of the disease depending on the severity.

CONCLUSIONS

The emergence of current outbreak of SARS-CoV-2 (COVID-19) pandemic is a reminder of how new pathogens can mutate, emerge and rapidly spread through large amount of human population, as well as cause severe burden to public health and healthcare system is general. Further research must be conducted to establish the fea-

Table II. Frequency of concomitant diseases in positive COVID19 cases in Zakarpattya during 2020

Presence of concomitant diseases					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Unknown	3363	10.1	10.1	10.1
	No	20270	61.1	61.1	71.2
	Yes	9557	28.8	28.8	100.0
	Total	33190	100.0	100.0	

Table III. Outcome of the COVID19 infection among positive cases in Zakarpattya during 2020.

Outcome of COVID19 infection					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid		4016	12.1	12.1	12.1
	Remains ill	573	1.7	1.7	13.8
	Unknown	1	.0	.0	13.8
	Recovery	27874	84.0	84.0	97.8
	Deceased	726	2.2	2.2	100.0
	Total	33190	100.0	100.0	

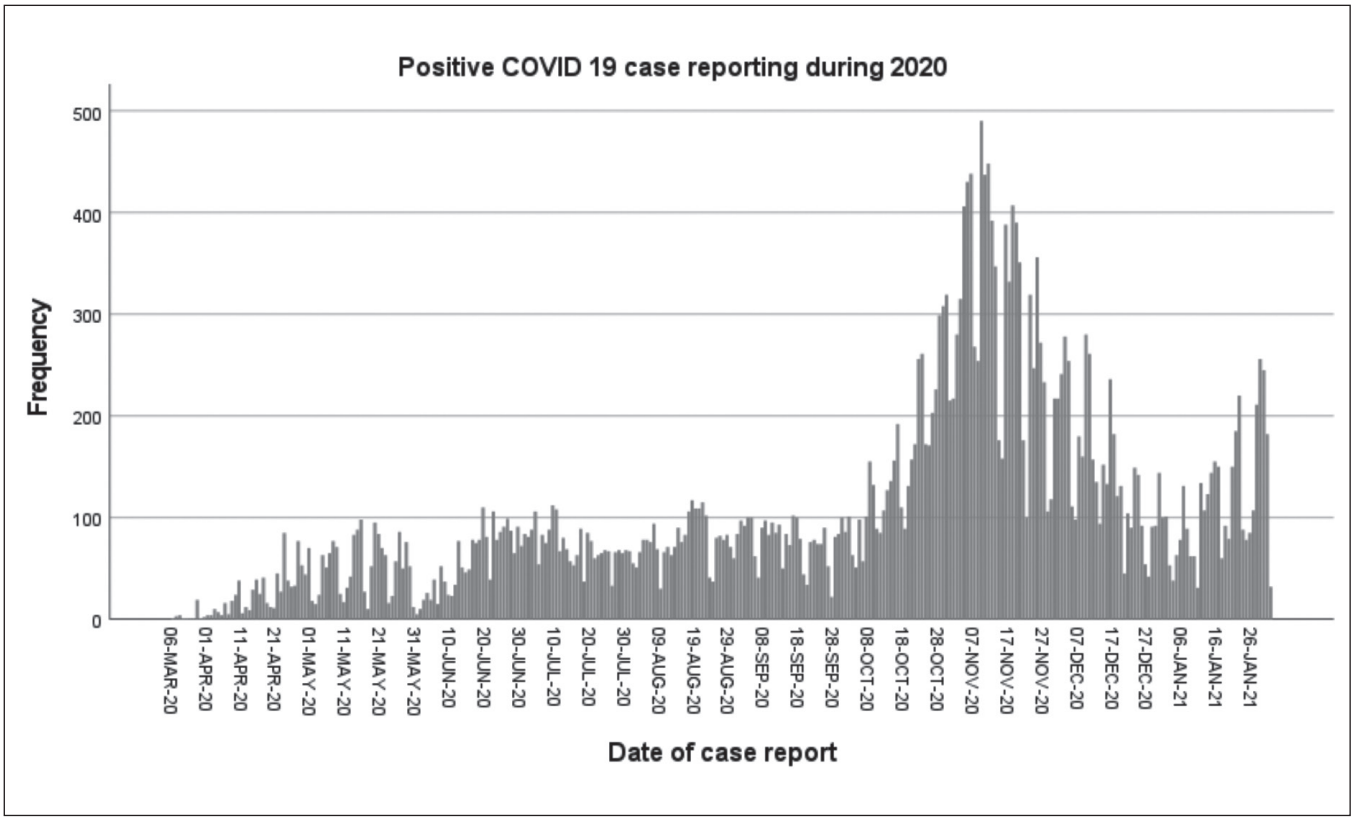

Fig. 2. Seasonal reporting of COVID19 cases in Zakarpattya during 2020.

Fig. 3. Geographical distribution of RT-PCR positive cases of Zakarpattya oblast by district

* Districts (rayon) of Zakarpattya oblast: 1 – Beregivsky, 2 -Velykobereznyansky, 3 – Vynogradivsky, 4 – Volovetsky, 5 – Irshavsky, 6 – Mizhgirsky, 7 –Mukachivsky, 8 – Perechynsky, 9 – Rahivsky, 10 – Svalyasky, 11 – Tyachisky, 12 – Uzhgorodsky, 13 – Hustsky, 14 – Chopsky.

tures of clinical manifestation of COVID-19 among hospitalized patients to better understand the natural history of the disease and its possible complications based on laboratory and instrumental findings. Given the fact that majority of the cases are treated with antibiotics, inpatient and recovered patients should be screened for the possible development of antibiotic resistance.

REFERENCES

1. Dhama K. et al. Coronavirus Disease 2019-COVID-19. Clin Microbiol Rev/ 2020;33(4).
2. Li Q., G.X., Wu P. et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. The New England journal of medicine. 2020;382(13): 1199-1207.
3. Nishiura H., Akhmetzhanov A.R. Initial Cluster of Novel Coronavirus (2019-nCoV) Infections in Wuhan, China Is Consistent with Substantial Human-to-Human Transmission. Journal of clinical medicine. 2020;9(2): 488.
4. Bchetnia M. et al. The outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): A review of the current global status. J Infect Public Health. 2020; 13(11): 1601-1610.
5. Koroutchev R. Economic situation and migration trends of Eastern Slovakia and Transcarpathian Ukraine. Journal of Liberty and International Affairs. 2020;5(3): 9-26.
6. Romero-Severson E.O. et al. Change in global transmission rates of COVID-19 through May 6 2020. PLoS One. 2020; 15(8).
7. Zheng R. et al. Spatial transmission of COVID-19 via public and private transportation in China. Travel Med Infect Dis. 2020; 34:101626.
8. Ministry of Health of Ukraine. Analytical panels and dashboards. 2020. https://covid19.gov.ua/analitichni-paneli-dashbordy.
9. Ke R. et al. Fast spread of COVID-19 in Europe and the US suggests the necessity of early, strong and comprehensive interventions. 2020. doi: 10.1101/2020.04.04.20050427.
10. Toyoshima Y. et al. SARS-CoV-2 genomic variations associated with mortality rate of COVID-19. Journal of Human Genetics. 2020;65:1075–1082.
11. Sanyaolu A. et al. Comorbidity and its Impact on Patients with COVID-19. SN Compr Clin Med. 2020: 1-8.

This project has been conducted as part of PhD research "Clinical and Epidemiological features of COVID19 patients hospitalized at the Regional Clinical Infectious Diseases Hospital during 2020".
All data used in this study can be accessed directly through the dashboard of the Public Health Center of the Ministry of Health of Ukraine [https://covid19.rnbo.gov.ua/], as well as on demand from Electronic Integrated Information System for Infectious Disease Surveillance (ELISS) with all parameter values provided in the text of the manuscript.

ORCID and contributionship:
Stepan O. Karabinyosh: 0000-0002-1252-7462 [A,B,C,D]
Galina M. Koval: 0000-0002-0623-2326 [D,E,F]

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Stepan O. Karabinyosh
Uzhhorod National University
148 Sobranetska st., 88015 Uzhhgorod, Ukraine
tel: +380953163221
e-mail:stepan.karabinyosh@uzhnu.edu.ua

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ORIGINAL ARTICLE

REVISITING THE VALUE OF HAEMATOLOGICAL AND BIOCHEMICAL MARKERS AND THE RATIOS IN PATIENTS WITH CORONARY ARTERY DISEASE

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OksanaYu. Marchenko[1,2], Nadiia M. Rudenko[1,2], Volodymyr V. Vitomskyi[1,3], Bohdana M. Habida[1]
[1]UKRAINIAN CHILDREN`S CARDIAC CENTER, KYIV, UKRAINE
[2]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[3]NATIONAL UNIVERSITY OF UKRAINE ON PHYSICAL EDUCATION AND SPORT, KYIV, UKRAINE

ABSTRACT

The aim: To investigate haematological and biochemical parameters and their potential for grading the severity of coronary artery lesions to predict the course atherosclerosis.

Materials and methods: The study is based on data obtained from a prospective analysis of 131 patients at the age of 51 to 82 years old from January to December 2019, whose complaints could indicate the coronary artery disease. All patients underwent a comprehensive clinical, laboratory and instrumental examination.

Results: The ratios of haematological and biochemical parameters significantly correlated with Syntax Score I. Some indexes did not confirm significant correlations with the severity of coronary artery disease. NT-proBNP, as a biochemical parameter, was the highest in patients with multi-vessel coronary artery disease and it had a moderate positive correlation with Syntax Score I ($r = 0.428$, $p = 0.0001$).

Conclusions: This study shows that ordinary indexes can be useful for assessment in daily practice for difficult patients. NTproBNP as an indicator requires further study as an additional marker for assessing the state of the cardiovascular system and can influence the choice of treatment.

KEY WORDS: ischemic heart disease (CHD), NT-proBNP, SYNTAX Score

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INTRODUCTION

Annually, cardiovascular disease (CVD) causes 3.9 million deaths in Europe and more than 1.8 million deaths in the European Union. Currently, CVD mortality is decreasing somewhat in most European countries, including Central and Eastern European countries that had shown substantial rises until the beginning of the 21st century [1]. Atherosclerosis is one of the main causes of CVD.

Pathophysiological mechanisms of atherosclerosis are still not well understood. Atherosclerosis is associated with inflammatory processes of the vascular wall. In the initial stage of atherosclerotic plaque formation, the vascular wall becomes permeable to both low-density lipoproteins (LDL) and leukocytes, namely neutrophils and monocytes, from the circulating blood [2]. Monocytes will differentiate into macrophages absorbing LDL, forming foam cells which subsequently will develop to lipid spots. Given the involvement of peripheral blood elements in the formation of atherosclerotic plaques, the investigation of haematological indices including lymphocyte-to-monocyte ratio [3, 4], platelet-to-lymphocyte ratio [5,6,7], neutrophil-to-lymphocyte ratio [8,9] and systemic immune-inflammatory index [10,11] is in active use.

Further, the progression of atherosclerosis is associated with elevated LDL-concentrations leading to higher risk of cardiovascular events . Cohen et al. demonstrated higher acute coronary event rates in individuals with PCSK9 mutations and low plasma LDL [12].

The imbalance of the lipid components has a significant impact on the development of atherosclerotic lesions and the progression of atherosclerosis. For example, the negative effect of reduced high-density lipoproteins (HDL) and elevated triglycerides has been proven [13]. The ratios of various fractions of lipid metabolism are also useful for predicting cardiovascular events.

THE AIM

The present study focuses on the value of haematological and biochemical parameters and their potential for grading the severity of coronary artery lesions to predict the course atherosclerosis.

MATERIALS AND METHODS

At the Ukrainian Children's Cardiac Center (Kyiv, Ukraine) 131 patients whose complaints were highly suggestive for coronary heart disease (CHD) were enrolled throughout 2019. Patients with connective tissue disease, hormone replacement therapy, severe valvular heart disease, hyper-

Table I. Patient's characteristics

Parameter	Group I (n= 30)	Group II (n= 35)	Group III (n= 66)	p-value
Age, years	60.53 ± 1.77	64.31 ± 1.62	63.0 ± 1.14	0.39
Male,%	30%	68.6%	78.8%	0.0001
BMI, kg/m2	31.74 ± 1.09	29.76 ± 0.77	30.71 ± 0.62	0.432
History of heart attack, n (%)	0	10 (28.6%)	33 (50%)	0.0001
History of stroke, n (%)	3 (10%)	5 (14.3%)	12 (18.2%)	0.576
Chronic Kidney Disease, n (%)	6 (20%)	7 (20%)	13 (19.7%)	0.999
Alcohol, n (%)	0	1 (2.9%)	5 (7.6%)	0.219
Smoking, n (%)	7 (23.3%)	11 (31.4%)	23 (34.8%)	0.529
Atrial fibrillation, n (%)	5 (16.7%)	5 (14.3%)	4 (6.1%)	0.215
COPD, n (%)	7 (23.3%)	1 (2.9%)	9 (13.6%)	0.049
Family history, n (%)	16 (53.3%)	16 (45.7%)	27 (40.9%)	0.523
Thyroid disease, n (%)	10 (33.3%)	8 (22.9%)	10 (15.2%)	0.127
Diabetes mellitus / impaired tolerance, n (%)	4 (13.3%)	7 (20%)	20 (30.3%)	0.388
Arterial hypertension, n (%)	30 (100%)	35 (100%)	66 (100%)	1.0
NYHA I, n (%)	-	9 (25.7%)	13 (19.7%)	0.49
NYHA II, n (%)	-	18 (51.4%)	37 (56.1%)	0.68
NYHA III, n (%)	-	8 (22.9%)	16 (24.2%)	0.87
Statins, n (%)	13 (43.3%)	25 (71.4%)	57 (86.4%)	0.0001
β-blockers, n (%)	17 (56.7%)	17 (48.6%)	49 (74.2%)	0.03
RAAS inhibitors, n (%)	16 (53.3%)	23 (65.7%)	52 (78.8%)	0.04
Calcium antagonists, n (%)	8 (26.7%)	10 (28,6)	19 (28.8%)	0.98
Syntax score I	-	5 (0;10)	27.75 (18; 38)	0.007

BMI – Body Mass Index, COPD - chronic obstructive pulmonary disease, NYHA - heart failure class according to the New York Association of Cardiologists, RAAS - renin-angiotensin-aldosterone system.

trophic cardiomyopathy, and pregnancy were excluded. The study was conducted in accordance with the World Medical Association's Code of Ethics (Declaration of Helsinki) and was approved by the Center Review Board. All subjects provided written informed consent.

Based on the findings of coronary angiography, the patients were divided into group I – control group without CHD (n=30), group II – single-vessel CHD or non-stenotic coronary atherosclerosis (n=35) or group III – multi-vessel CHD (n=66).

All groups were comparable with respect to age, body mass index (BMI) and comorbidities (Tab.I). However, the control group consisted of a higher number of females and a higher percentage of patients with chronic obstructive pulmonary disease. Between group II and III no statistically significant differences were observed for NYHA class.

All patients underwent clinical examination and laboratory testing. Venous blood samples were taken from each subject, and were subsequently centrifuged prior to testing. Blood count analyses (leucocyte- [WBC], neutrophil-, lymphocyte-, monocyte-, red blood- [RBC] and platelet count [PLT]) as well as hemoglobin [Hb] and haematocrit measurements were performed using an ABX Pentra 60 C+ hematology analyzer («HORIBA ABX» – Montpellier, France). For complete blood count and ESR analyses, venous blood samples were taken from each patient and were collected in blood tubes containing ethylenediaminetetraacetic acid or citrate. Biochemical analyseis (total protein, C-reactive protein [CRP], fasting blood glucose, alanine aminotransaminase [ALT], aspartate aminotransferase [AST], blood urea, creatinine, total bilirubin, calcium [Ca], potassium [K], sodium [Na], fasting triglyceride [TG], total cholesterol [TC], low density lipoprotein- cholesterol [LDL-C], and high density lipoprotein-cholesterol [HDL-C]) were performed using an AU 480 chemistry analyzer (Beckman Coulter – Brea, California, United States). All tests were performed according to the manufacturers' instructions. The level of the N-terminal fragment of the brain natriuretic peptide precursor (NT-proBNP) was analyzed with enzyme-linked fluorescence assay by automatic miniVIDAS® (bioMerieux – Craponne, France). Samples for coagulation tests which included prothrombin index (PTI) and international normalization ratio were investigated by Thrombotimer 4 (Behnk Elektronik – Norderstedt, Germany).

Statistical analyses were performed using Excel 2010 and SPSS Statistics 20.0. Both, parametric and non-parametric (Shapiro-Wilk test) data were presented as mean values with

Table II. Biochemical parameters

Parameter	Group I (n= 30)	Group II (n= 35)	Group III (n= 66)	p-value
Haemoglobin, g/L	139.5 (127.0; 152.0)	148.0 (133.0;157.5)	150.5 (142.0;158.0)	0.06
RBC,1012/L	4.65 (4.20; 5.00)	4.80 (4.35; 5.00)	4.90 (4.70; 5.10)	0.09
ESR, mm/h	9.5 (4.0; 19.0)	11.0 (7.0; 16.5)	12.0 (7.0; 22.0)	0.69
Haematocrit, %	42.0 (39.0; 46.0)	45.0 (40.0; 47.0)	46.0 (43.0; 47.0)	0.07
PLT, 109/L	225.5 (196.0; 266.0)	232.0 (197.5; 266.0)	237.5 (192.0; 285.0)	0.53
WBC, 109/L	5.7 (4.9; 6.8)	6.9 (5.8; 7.6)	7.2 (6.3; 7.9)	0.007
Neutrophils, 109/L	3.34 (2.35; 4.09)	3.95 (3.35; 5.21)	4.20 (3.47; 5.03)	0.003
Lymphocytes, 109/L	1.91 (1.37; 2.21)	1.73 (1.42; 2.20)	2.0 (1.61; 2.58)	0.11
Monocytes, 109/L	0.48 (0.28; 0.60)	0.51 (0.39; 0.71)	0.53 (0.42; 0.65)	0.12
Total protein, g /L	73.25 (70.30; 78.90)	72.90 (70.80; 75.60)	73.20 (70.20; 76.00)	0.587
Glucose, mmol / L	6.0 (5.2; 7.3)	5.8 (5.5; 6.5)	6.2 (5.6; 7.5)	0.212
Urea, mmol /L	6.0 (5.3; 7.3)	5.7 (5.3; 6.9)	6.2 (5.2; 7.8)	0.647
Creatinine, μmol /L	94.55 (82.30; 104.90)	95.30 (83.00; 105.05)	106.35 (96.10; 122.10)	0.001
GFR by MDRD, mL/min /1.73 m2	63.00 (52.00; 73.00)	68.00 (60.50; 77.00)	60.50 (52.00; 71.00)	0.204
Total bilirubin, μmol/L	14.15 (11.80; 19.40)	14.20 (12.25; 21.15)	12.85 (11.50; 17.20)	0.268
ALT, U/L	21.85 (16.40; 35.70)	22.80 (17.95; 32.35)	27.40 (18.40; 42.10)	0.225
AST, U/L	19.85 (16.70; 23.90)	21.20 (18.20; 26.25)	23.10 (18.10; 31.40)	0.132
NT-proBNP, pg/mL	99.00 (42.70; 135.10)	121.60 (71.65; 201.50)	299.35 (96.60; 578.00)	0.0001
CRP, mg /L	3.95 (2.30; 6.70)	2.80 (1.81; 7.00)	4.30 (2.00; 10.10)	0.397
K+, mmol/L	4.20 (4.00; 4.60)	4.20 (4.05; 4.60)	4.30 (4.00; 4.60)	0.808
Na+, mmol/L	141.00 (139.00; 142.00)	140.00 (138.00; 142.00)	140.00 (138.00; 143.00)	0.421
Ca2+, mmol/L	1.23 (1.19; 1.25)	1.21 (1.20; 1.26)	1.24 (1.20; 1.28)	0.188
PTI, %	85.00 (78.00; 93.00)	85.00 (76.00; 94.50)	82.00 (75.00; 90.00)	0.206
INR	1.10 (1.05; 1.15)	1.10 (1.04; 1.18)	1.13 (1.07; 1.18)	0.157

Data are represented as mean values - M ± m and median with 25th and 75th percentiles - Me (25–75%). RBC – red blood count, ESR - erythrocyte sedimentation rate, WBC - leukocyte count, PLT - platelet count, GFR by MDRD - glomerular filtration rate according to the MDRD equation, ALT - alanine aminotransferase, AST - aspartate aminotransferase, NT-proBNP - N-terminal pro–B-type natriuretic peptide, CRP - C-reactive protein, K – potassium, Na - sodium, Ca - calcium, PTI - prothrombin index, INR - International Normalized Ratio.

Table III. Lipidogram

Parameter	Group I (n= 30)	Group II (n= 35)	Group III (n= 66)	p-value
Cholesterol, mmol/L	5.00 (4.50; 5.80)	4.30 (3.45; 5.20)	4.15 (3.50; 5.00)	0.008
Triglycerides, mmol/L	1.14 (0.74; 1.69)	1.10 (0.78; 1.29)	1.36 (1.05; 2.04)	0.002
HDL, mmol/L	1.17 (1.12; 1.46)	1.08 (0.96; 1.27)	1.02 (0.86; 1.14)	0.0001
LDL, mmol/L	3.26 (2.41; 3.75)	2.58 (1.97; 3.34)	2.49 (1.92; 3.23)	0.014
Non-HDL, mmol/L	3.73 (3.24; 4.45)	3.27 (2.45; 4.00)	3.15 (2.46; 3.92)	0.029
HDL / Cholesterol	4.02 (3.53; 4.57)	3.82 (3.22; 4.53)	4.18 (3.33; 5.00)	0.241
TG / HDL	0.92 (0.59; 1.53)	0.92 (0.61; 1.26)	1.48 (1.00; 2.08)	0.0001
LDL / HDL	2.54 (1.97; 3.14)	2.26 (1.84; 2.90)	2.44 (1.85; 3.13)	0.417
Non-HDL / HDL	3.02 (2.53; 3.57)	2.82 (2.22; 3.53)	3.18 (2.33; 4.00)	0.241

Data are represented as mean values - M ± m and median with 25th and 75th percentiles - Me (25–75%).

Table IV. Hematological and biochemical indices

Parameter	Group I (n= 30)	Group II (n= 35)	Group III (n= 66)	p-value
Monocyte-to-HDL (10*9 / mmol /L)	0.34 (0.20; 0.51)	0.44 (0.32; 0.70)	0.55 (0.39; 0.71)	0.002
Neutrophil-to-HDL (10*9 / mmol /L)	2.66 (1.89; 3.30)	3.39 (2.70; 4.80)	4.30 (3.36; 5.03)	0.0001
Lymphocyte-to-HDL (10*9// mmol / L)	1.45 (1.1; 1.93)	1.67 (1.30; 2.15)	1.97 (1.52; 2.85)	0.002
Lymphocyte-to-monocyte (10*9)	4.27 (2.80; 6.67)	3.63 (2.79; 4.86)	4.15 (2.73; 5.25)	0.176
Platelets-to- lymphocytes (10*9)	116.58 (92.03; 153.52)	129.42 (100.29; 176.26)	120.29 (92.11; 158.41)	0.552
Neutrophil-to- lymphocytes (10*9)	1.65 (1.44; 2.48)	2.07 (1.66; 3.19)	2.02 (1.50; 2.72)	0.131
SII	369.54 (243.28; 627.43)	450.95 (358.96; 777.90)	369.12 (502.03; 645.65)	0.077

SII - systemic immuno-inflammation index

standard deviation (M ± m) and as median with 25th and 75th percentiles (Me (25–75%)). Pearson χ^2 and Kraskel-Wallis tests were used to assess significance between two independent samples. Spearman and Pearson's correlation coefficient were used for ratio assessment. Statistical significance was considered with a two-sided p-value < 0.05.

RESULTS

Haematology test panel showed no differences for haemoglobin, erythrocytes, erythrocyte sedimentation rate (ESR), haematocrit, platelets, lymphocyte and monocyte levels between all groups (Tab.II). In contrast, significant differences were observed for leukocyte (p = 0.007) and neutrophil (p = 0.003) levels with the highest rates in group III. Medians of these values were within normal ranges in all groups. Biochemical blood assay revealed a significantly higher level of creatinine in group III, but glomerular filtration rate (GFR) by MDRD did not differ significantly between the groups (p = 0.204, Tab.II).

The NT-proBNP level was significantly higher in group III (median: 299.35 pg/mL (96.60; 578.00), p = 0.0001; reference value: <125 pg/mL) [14]. NT-proBNP levels correlate with the patients' prognosis and is recommend for patients with acute or chronic heart failure to verify the diagnosis. Ce Zhang et al. studied NT-proBNP as an

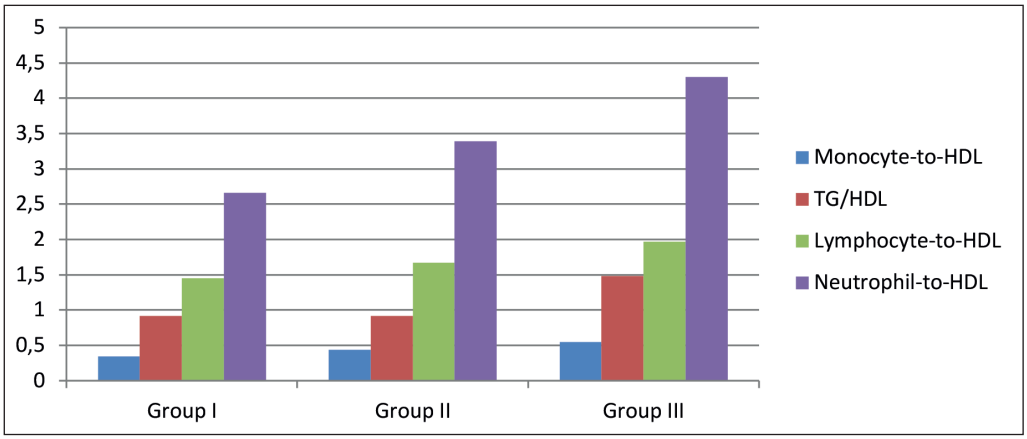

Fig 1. Shows the ratios that were significantly correlated with the degree of CHD according to Syntax Score I.

additional biomarker for risk stratification and therapeutic decision-making in patients with three-vessel lesions [15]. The results of our study demonstrated a moderate positive correlation with Syntax Score I (r = 0.428, p = 0.0001, Tab.II).

The lipidograms were significantly different between all groups. Group III had the lowest total cholesterol, probably due to statin intake because group III showed the highest percentage of patients receiving statins (86.4%).

Triglyceride levels were in line with the recommended standards of the ESC Guidelines: <1.7 mmol /L [16]. Highest triglyceride levels were observed in group III: 1.36 (1.05; 2.04) mmol /L whereas group II showed the lowest levels: 1.10 (0.78; 1.29) mmol /L.

The highest level of HDL-C was found in controls: 1.17 (1.12; 1.46) mmol /L, the lowest in group III: 1.02 (0.86; 1.14) mmol /L. LDL-C was increased in all groups. The highest level was observed in group I: 3.26 (2.41; 3.75) mmol /L exceeding the recommended limits, although all patients were classified as low risk patients [15]. In high risk patients with multi-vessel CHD (group III) a LDL-C target of <1.4 mmol/L is recommended [15]. Although 86% of patients in group III were on statins, the median of 2.49 (1.92; 3.23) mmol/L indicates an underestimation of the cardiovascular risk with probably insufficient statin dose.

Non-HDL values were not within target ranges, either: groups I: 3.73 (3.24; 4.45) mmol/L, groups II: 3.27 (2.45; 4.00) mmol/L and groups III: 3.15 (2.46; 3.92) mmol/L.

While evaluating the ratios of various lipid fractions, a significant difference was only seen in TG-to-HDL ratios (p = 0.0001). In group III, this ratio was more than 1.5 times higher than in groups I and II and there was a moderate positive correlation with Syntax Score S scores (r = 0.306, p = 0.0001, median 1.48 (1.00; 2.08); Tab.III).

High-density lipoproteins are used to calculate indices with haematological parameters, such as the number of lymphocytes, monocytes and neutrophils. These indices are classified as markers of inflammatory processes. Mehmet Serkan Cetin at al. reported that a higher monocyte-to-HDL ratio is a negative predictor for the development of acute coronary syndrome (ACS) and the severity of CHD [17]. In comparison to neutrophil-to-lymphocyte ratio and C-reactive protein, monocyte-to-HDL ratio might be superior predicting the severity of CHD in patients with ACS who underwent percutaneous coronary interventions [18]. According to the present data, monocyte-to-HDL ratio was significantly higher in patients with multi-vessel CHD (p = 0.002) and showed a moderate positive correlation with Syntax Score I (r = 0.301, p = 0.0001; Tab.IV).

Furthermore, neutrophil-to-HDL and lymphocyte-to-HDL ratios were significantly higher in group III (p = 0.0001 and p = 0.002, respectively) being consistent with the data reported by Jia-Bao Huang et al., who demonstrated that this ratio might be a useful predictor of long-term clinical outcomes in the elderlies with acute myocardial infarction [19].

DISCUSSION

1. The level of triglycerides was significantly higher in group III (p = 0.002), but in all groups the value was <1.7 mmol /l. The level of high-density lipoprotein (HDL) was the highest in the control group (p = 0.0001). Non-HDL was the lowest in group III, but its level in all groups was not target (p = 0.029).

One of the reasons for this is the non-appointment of statin therapy in patients with high and very high risk. Thus, März W. et al.- [20] notes that in a cohort of patients (more than 42,000 patients) the percentage of hypolipidemic therapy remains quite low at 35%. All patients had proven atherosclerotic lesions and risk factors such as diabetes. Achieving LDL targets had low ranges (from 26.9% to 46.7%). One more reason for not enough reduction is the choice of insufficient dose or medication. According to STELLAR Trial [21], rosuvastatin outperformed other drugs significantly and the percentage of achievement of target values for LDL ranged from 82 to 89%. On the other hand, LDL reduction by 50% is the option as a goal, not only to achieve absolute numbers; an individualized approach should remain a priority for the cardiologist.

2. The ratios of haematological and biochemical parameters that significantly correlated with Syntax Score I were as follows: monocyte-to-HDL (r = 0.400, p = 0.0001) and TG-to-HDL (r = 0.306, p = 0.0001), lymphocyte-to-HDL (r = 0.336, p = 0.0001), and neutrophil-to-HDL (r = 0.400, p = 0.0001). The results of monocyte-to-HDL coincide with

the data of Akboga MK. et al [22] who notes that this index is associated with a more significant coronary lesion by the number of points on the SYNTAX scale, namely ≥23.

Li Y. et al. [23] demonstrated that elevated TG-to-HDL index is one of the potential indicators of stent stenosis after PCI. In our work it is shown that this index positively correlates with the degree of damage on the SYNTAX score. It should be noted that the increased level of triglycerides, as a component of this index, plays an important role in the progression of atherosclerosis [24].

The lymphocyte-to-HDL and neutrophil-to-HDL indices are not widely described in the literature. These markers are studied in metabolic syndrome in some articles [25]. In our work, it has been shown for the first time that both markers have a moderate positive correlation with the degree of coronary artery disease, namely the quantitative characteristic in points on the SYNTAX score. These markers need further investigation.

The given data testify to indisputable expediency of the use of indices in the practice of the doctor. These parameters to calculate are simple and can be useful for risk estimation by a Heart team for decision-making.

3. NT-proBNP levels were the highest in patients with multi-vessel coronary artery disease, and they had a moderate positive correlation with Syntax Score I ($r = 0.428$, $p = 0.0001$). Recently, there has been growing evidence for decision-making on intervention (CABG, PCI) that requires a quantitative assessment of the presence and extent of hemodynamic stress and manifestations of heart failure. NT-proBNP concentration is used for this purpose [26].

The increased concentration of this marker may provide additional prognostic information for adverse effects, including death, myocardial infarction, and stroke [27]. There is an assumption that at a normal level of NT-proBNP even insignificant increase is an indicator for the necessity to give preference to PCI. At the same time, CABG is recommended when this marker increases significantly[15]. It can be beneficial as an additional parameter for patients with coronary artery disease and may influence a choice of treatment (PCI, CABG).

4. There was no significant difference in lymphocyte-to-monocyte, platelet-to-lymphocyte, neutrophil-to-lymphocyte ratio, or systemic immuno-inflammation index between the groups. The above values did not correlate with the degree of coronary artery disease on the Syntax Score Scale, either. Therefore, these markers need further study to address their prognostic value.

CONCLUSIONS

This study shows ordinary indexes can be useful for assessment in daily practice for difficult patients, but our investigation did not show the significance of some of them such as lymphocyte-to-monocyte, platelet-to-lymphocyte, neutrophil-to-lymphocyte ratio, or systemic immuno-inflammation index.

Further research is useful and undeniable for NTproBNP as an independent marker of more significant coronary vessels lesions. It can be used as a predictor of not only more significant vascular lesion but also assessment for treatment after PCI and CABG.

REFERENCES

1. European Cardiovascular Disease Statistics. 2017. http://www.ehnheart.org/cvd-statistics.html.
2. Nguyen M.T., Fernando S., Schwarz N. et al. Inflammation as a Therapeutic Targetin Atherosclerosis. J. Clin. Med. 2019;8:1109. https://doi.org/10.3390/jcm8081109.
3. Liu Si.Y., Shan W.J. et al. Association of lymphocyte-to-monocyt ratio with total coronary plaque burdening patients with coronary artery disease. Coronary Artery Disease. 2020; 31(7):650-655.
4. Gong S., Gao X., Xu F. et al. Association of lymphocyte to monocyte ratio with severity of coronary artery disease. Medicine (Baltimore). 2018;97(43):e12813. doi:10.1097/MD.0000000000012813.
5. Parlar H., Şaşkın H. Are Pre and Postoperative Platelet to Lymphocyte Ratio and Neutrophil to Lymphocyte Ratio Associated with Early Postoperative AKI Following CABG? Braz J Cardiovasc Surg. 2018;33(3):233-241. doi: 10.21470/1678-9741-2017-0164.
6. Serrano C.V., de Mattos F.R., Pitta F.G. et al. Association between Neutrophil-Lymphocyte and Platelet-Lymphocyte Ratios and Coronary Artery Calcification Score among Asymptomatic Patients: Data from a Cross-Sectional Study. Mediators Inflamm 2019;651384. https://doi.org/10.1155/2019/6513847.
7. Bressi E., Mangiacapra F., Ricottini E. et al. Impact of Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio on 5-Year Clinical Outcomes of Patients with Stable Coronary Artery Disease Undergoing Elective Percutaneous Coronary Intervention. J Cardiovasc Transl Res. 2018;11(6):517-523. doi: 10.1007/s12265-018-9829-6.
8. Angkananard T., Anothaisintawee T., McEvoy M. et al. Neutrophil Lymphocyte Ratio and Cardiovascular Disease Risk: A Systematic Review and Meta-Analysis. Biomed Res Int [Internet]. 2018;2703518. https://doi.org/10.1155/2018/2703518.
9. Afari M.E., Bhat T. Neutrophil to lymphocyteratio (NLR) and cardiovascular diseases: anupdate. ExpertRevCardiovascTher. 2016;14(5):573-7. doi: 10.1586/14779072.2016.1154788.
10. Yang Y.L., Wu C.H., Hsu P.F. et al. Systemic immune-inflammation index (SII) predicted clinical outcome in patients with coronary artery disease. Eur J ClinInvest. 2020;50(5):e13230. doi: 10.1111/eci.13230.
11. Dey S., Kashav R., Kohli J.K. et al. Systemic Immune-Inflammation Index Predicts Poor Outcome After Elective Off-Pump CABG: A Retrospective, Single-Center Study. J Cardiothorac Vasc Anesth. 2020:S1053-0770(20)31007-7. doi: 10.1053/j.jvca.2020.09.092.
12. Cohen J.C., Boerwinkle E., Mosley T.H. et al. Variations in PCSK9, Low LDL, and Protection against Coronary Heart Disease. N Engl J Med [Internet]. 2006;354(12):1264–72. https://doi.org/10.1056/NEJMoa054013.
13. Toth P.P, Granowitz C., Hull M. et al. High Triglycerides Are Associated With Increased Cardiovascular Events, Medical Costs, and Resource Use: A Real–World Administrative Claims Analysis of Statin–Treated Patients With High Residual Cardiovascular Risk. J Am Heart Assoc [Internet]. 2018;7(15):e008740. doi: 10.1161/JAHA.118.008740.
14. Ponikowski P., Voors A.A., Anker S.D. et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Heart Journal, 2016;37(27):2129–2200.

15. Zhang C., Jiang L., Xu L. et al. Implications of N-terminal pro-B-type natriuretic peptide in patients with three-vessel disease, European Heart Journal, 2019;40(41):3397–3405 https://doi.org/10.1093/eurheartj/ehz394.
16. Mach F., Baigent C., Catapano A.L. et al. ESC Scientific Document Group. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41(1):111-188. doi: 10.1093/eurheartj/ehz455.
17. Cetin M.S., Ozcan Cetin E.H., Kalender E. et al. Monocyte to HDL Cholesterol Ratio Predicts Coronary Artery Disease Severity and Future Major Cardiovascular Adverse Events in Acute Coronary Syndrome. Heart Lung Circ. 2016;25(11):1077-1086. doi: 10.1016/j.hlc.2016.02.023.
18. Çağdaş M., Karakoyun S., Yesin M. et al. The Association between Monocyte HDL-C Ratio and SYNTAX Score and SYNTAX Score II in STEMI Patients Treated with Primary PCI. Acta Cardiol Sin. 2018;34(1):23-30. doi: 10.6515/ACS.201801_34(1).20170823A.
19. Huang J.B., Chen Y.S., Ji H.Y. et al. Neutrophil to high-density lipoprotein ratio has a superior prognostic value in elderly patients with acute myocardial infarction: a comparison study. Lipids Health Dis. 2020;19(1):59. doi: 10.1186/s12944-020-01238-2.
20. März W., Dippel F.W., Theobald K. et al. Utilization of lipid-modifying therapy and low-density lipoprotein cholesterol goal attainment in patients at high and very-high cardiovascular risk: Real-world evidence from Germany. Atherosclerosis. 2018;268:99-107. doi: 10.1016/j.atherosclerosis.2017.11.020.
21. Jones P.H., Davidson M.H., Stein E.A. et al. STELLAR Study Group. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). Am J Cardiol. 2003;92(2):152-60. doi: 10.1016/s0002-9149(03)00530-7.
22. Akboga M.K., Balci K.G., Maden O. et al. Usefulness of monocyte to HDL-cholesterol ratio to predict high SYNTAX score in patients with stable coronary artery disease. Biomark Med. 2016;10(4):375-83. doi: 10.2217/bmm-2015-0050.
23. Li Y., Jin P., Hou F. et al. Association Between TG-to-HDL-C Ratio and In-Stent Stenosis Under Optical Coherence Tomography Guidance. J Med Syst. 2018;43(1):4. doi: 10.1007/s10916-018-1119-y.
24. Generoso G., Janovsky S., Bittencourt M.S. Triglycerides and triglyceride-rich lipoproteins in the development and progression of atherosclerosis. Curr Opin Endocrinol Diabetes Obes. 2019;26(2):109-116. doi: 10.1097/MED.0000000000000468.
25. Chen T., Chen H., Xiao H. et al. Comparison of the Value of Neutrophil to High-Density Lipoprotein Cholesterol Ratio and Lymphocyte to High-Density Lipoprotein Cholesterol Ratio for Predicting Metabolic Syndrome Among a Population in the Southern Coast of China. Diabetes Metab Syndr Obes. 2020;13:597-605. doi: 10.2147/DMSO.S238990.
26. Redfors B., Chen S., Crowley A. et al. B-type natriuretic peptide assessment in patients undergoing revascularization for left main coronary artery disease. Circulation 2018;138:469–478
27. Michou E., Fahrni G., Mueller C. Quantifying heart failure using natriuretic peptides may help the HEART team in decision-making, European Heart Journal, 2019;40 (41):3406–3408. https://doi.org/10.1093/eurheartj/ehz572.

ORCID and contributionship:
OksanaYu. Marchenko: 0000-0003-4909-8347 [A,B,C,D]
Nadiia M. Rudenko: 0000-0002-1681-598X [E,F]
Volodymyr V. Vitomskyi: 0000-0002-4582-6004 [B,C]
Bohdana M. Habida: 0000-0003-1966-9575 [B]

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CORRESPONDING AUTHOR
Oksana Yu. Marchenko
Ukrainian Children`s Cardiac Center,
24 Yuriia Illenka St., 04050 Kyiv, Ukraine
tel: +38 0967613257
e-mail: marchenko.ox@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

AGGRAVATING EFFECT OF ARTERIAL HYPERTENSION ON THE COURSE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE IN PATIENTS WITH COMORBID PATHOLOGY

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Viktoriia V. Rodionova, Olha O. Boiko
STATE INSTITUTION «DNIPROPETROVSK MEDICAL ACADEMY OF THE MINISTRY OF HEALTH OF UKRAINE», DNIPRO, UKRAINE

ABSTRACT

The aim: To study the effect of arterial hypertension on the course of chronic obstructive pulmonary disease in patients with comorbid pathology.

Materials and methods: The prospective study included 61 patients with COPD: 32 stable male patients with COPD with comorbid arterial hypertension of stage II 1-3 degrees and 29 stable outpatients of men with COPD of clinical groups A-D with impaired respiratory function II-IV according to GOLD. All patients, in accordance with the goals and objectives of the study, were divided into 2 groups: group I consisted of men with isolated COPD, middle age – 56.0 (8.5) years, average duration of the disease – 16.2 (1.3) years, Group II consisted of male patients with COPD and arterial hypertension (AH), middle age – 59.5 (7.5). The patients underwent a general clinical examination, which included an assessment of complaints, anamnestic data, and a physical examination. The severity of COPD was determined on the basis of the frequency of exacerbations during the year, assessment of dyspnea using the mMRS scale, spirographic data. Statistical materials were processed using the STATISTICA 10.0 program.

Results: In group I, 20 patients (69%) complained of dyspnea during exercise, in group II – 25 patients (78%) ($p = 0.4$), 28 patients (96.5%) complained of cough with vague sputum. group and 30 patients in group II (93.8%) ($p = 0.09$). When assessing the number of exacerbations over the past year, it was determined that patients with isolated COPD had an average of 1.0 (1.0; 2.0) exacerbations, and patients with COPD and AH – 2.0 (1.0; 3.0) ($p = 0.06$). According to the CAT questionnaire, the following data were obtained: in group I – 9.0 (8.0; 11.0) points, and in group II – 17.5 (10.0; 20.0) points ($p = 0.02$). When conducting spirographic studies, a statistically significant more expressive bronchial obstruction was found in patients with COPD and comorbid hypertension.

Conclusions: The presence of comorbid arterial hypertension leads to the intermittent effect of diseases: according to the results of mMRC and SAT test, ailments for COPD were examined, they have a more severe course of underlying seizure in the presence of concomitant arterial hypertension. Clinical manifestations in patients with COPD and H are more severe compared to clinical manifestations in patients without aggravated diseases of the cardiovascular system. Concomitant arterial hypertension enhances the manifestations of bronchial obstruction, in the same way as with patients with isolated COPD.

KEY WORDS: hypertension, COPD, comorbid pathology

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INTRODUCTION

Polymorbidity is one of the features of the modern clinical picture of internal diseases, and arterial hypertension (AH) and chronic obstructive pulmonary diseases remain the most common diseases of the adult population of developed countries [1]. One of the most common nosological forms is chronic obstructive pulmonary disease (COPD) [2,3]. According to WHO experts, by 2030, COPD will become the third leading cause of death worldwide [2,4].

COPD is a chronic disease with a progressive course, which ranks third in the structure of causes of death in the world according to WHO. In 2016, there were 251 million cases of COPD worldwide, and in 2015 – 3,170,000 deaths of patients with COPD, which is 5% of all deaths in the world. More than 90% of deaths from COPD occur in low- and middle-income countries [1,2].

The main target organs in COPD are the bronchi and lungs, but at a certain stage in the development of the disease, other organs and systems are affected. The most significant correlation was found between COPD and cardiovascular disease. It has been established that COPD is a precursor to the development of cardiovascular diseases and / or its exacerbation [2,3,4,5]. It was found that there is a relationship between an increase in arterial wall stiffness and the severity of bronchial obstruction in patients, and a decrease in FEV1 is associated with a high risk of cardiovascular mortality [2,5]. Over the past 30 years, the spread of cardiovascular diseases in Ukraine has increased 3.5 times, and the mortality rate from them – by 46%. In the structure of morbidity, the first are arterial hypertension (AH) – 41% and ischemic heart disease – 28% [6,7,8]. The prevalence of arterial hypertension is also significantly higher in patients with COPD [7,2]. The common mechanisms of development of many extrapulmonary manifestations are chronic systemic inflammation, oxidative stress, and endothelial dysfunction [9]. The combination of CVD and COPD increases the pathological changes inherent in each disease separately, significantly changes the clinical picture, course and consequences of each of the associated diseases, leads to a significant complication [10].

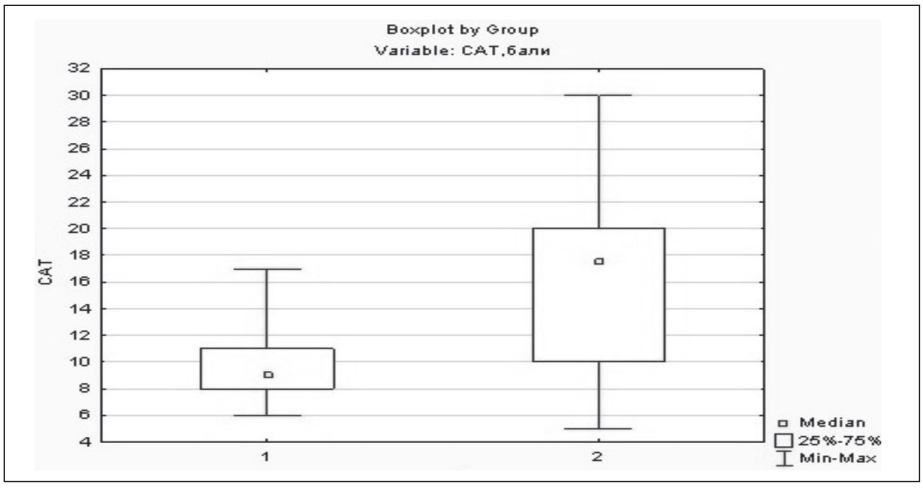

Fig. 1. Results of the CAT questionnaire in patients

Table 1. Respiratory function indicators in patients.

	Group I	Group II	p
FVC, % Me [25 %-75 %]	98,0(89,0;115,0)	92,0(59,0;108,0)	0,2
FEV_1, % Me [25 %-75 %]	73,0 (50,0;91,0)	44,0 (32,0;65,0)	0,03
FEV_1%M, Me [25 %-75 %]	66,0(36,0;77,4)	48,0(25,8;75,0)	0,04
MEF 75, % Me [25 %-75 %]	78,0(48,0;89,0)	26,0(20,0;67,0)	0,02
MEF 50, % Me [25 %-75 %]	73,0(10,0;76,0)	23,0(8,0;60,0)	0,02
MEF 25, % Me [25 %-75 %]	22,0(11,0;36,0)	41,0(18,0;62,0)	0,6
PEF, % Me [25 %-75 %]	75,0(56,0;89,0)	42,0(22,0;59,0)	0,5
IC_F, % Me [25 %-75 %]	43,0(40,0;92,0)	62,0(45,0;87,0)	0,8

THE AIM

To investigate the effect of arterial hypertension on the course of chronic obstructive pulmonary disease.

MATERIALS AND METHODS

The prospective study included 61 patients with COPD: 29 stable outpatients of men with COPD of clinical groups A-D with impaired respiratory function II – IV according to GOLD and 32 stable male patients with COPD with comorbid arterial hypertension of II stage 1-3 degrees. The patients were treated at the municipal non-profit institution «City Clinical Hospital No. 4» of the Dnipropetrovsk City Council during 2018-2020. The criteria for the inclusion of patients in the study were: the clinical diagnosis of COPD was verified in accordance with the recommendations of GOLD 2019 [2], the diagnosis of arterial hypertension was verified based on the recommendations for the treatment of arterial hypertension of the European Society of Cardiology and the European Society of Arterial Hypertension, 2018 [8], informed consent of patients for inclusion in research and processing of personal data. The exclusion criteria from the study were: the age of patients over 80 years old, a history of acute cardiovascular events, clinically significant cardiac arrhythmias, previously diagnosed diabetes mellitus, kidney disease, malignant neoplasms, and undergoing surgery over the last year. All patients, in accordance with the goals and objectives of the study, were divided into 2 groups: group I consisted of men with isolated COPD, middle age – 56.0 (8.5) years, average duration of the disease – 16.2 (1.3) years Group II included men with COPD and arterial hypertension (AH), middle age – 59.5 (7.5), with an average duration of COPD – 15.9 (1.7) years. Both groups were comparable in age and duration of COPD ($p > 0.05$). In group I, 3 patients belonged to category A COPD, 11 patients to category B, 13 patients to category C, and 2 patients to category D. In group II, 2 patients had COPD category A, 14 – category B, 14 – category C and 2 patients of category D. Patients underwent a general clinical examination, which included an assessment of complaints, anamnestic data, and a physical examination. The severity of COPD was determined based on the frequency of exacerbations during the year, assessment of dyspnea using the mMRS scale (The modified medical research counsil dyspnea scale) and COPD assessment test, spirographic data (forced expiratory volume in 1 second (FEV1), forced vital lung capacity (FVC), their ratio – FEV1 / FVC). The results of the study were processed using statistical methods. To identify the relationship between the indicators, a correlation analysis of paired measurements was carried out using the method of K. Pearson's coefficient ($\chi 2$), since the data had a distribution other than normal. Those indicators in which the level of disagreement was $p < 0.05$ were considered significant. The material was calculated using the Microsoft Excel 2000 software package, the statistical processing of the research materials was performed on a personal computer using the STATISTICA 10.0 licensed software (StatSoft Inc., Serial No. AGAR909E415822FA) [11].

RESULTS AND DISCUSSION

In group I, 20 patients (69%) complained of dyspnea during exercise, in group II – 25 patients (78%) (p = 0.4), 28 patients (96.5%) complained of cough with vague sputum. group and 30 patients in group II (93.8%) (p = 0.09). When assessing the number of exacerbations over the past year, it was determined that patients with isolated COPD had an average of 1.0 (1.0; 2.0) exacerbations, and patients with COPD and AH – 2.0 (1.0; 3.0) (p = 0.06). Despite the fact that the level of obstruction is not always correlated with the severity of symptoms in patients, the CAT and mMRC questionnaire was used [12].

The severity of dyspnea (mMRC scale) in patients of group I was 1.5 (1.0; 2.0) points, in patients of group II – 2.0 (1.0; 3.0) points (p = 0.07). Based on the data of the CAT questionnaire, a more complete scoring of respiratory symptoms and their impact on the life of patients was carried out, so in group I received 9.0 (8.0; 11.0) points, and in group II – 17.5 (10 0; 20.0) points (p = 0.02) (Fig. 1).

When conducting spirographic studies, a statistically significant more pronounced bronchial obstruction was found in patients with COPD and comorbid hypertension (Table 1).

The results obtained indicate a more severe course of COPD in the presence of comorbid arterial hypertension, which, in turn, has a greater impact on the quality of life of patients. The results obtained are comparable with the data of the ECLIPSE study, according to which the presence of hypertension in patients with COPD was associated with more severe dyspnea [13].The results obtained in our study are confirmed by the data of Caram L. M., Ferrari R., Naves C. R., which indicate that patients with concomitant hypertension have more severe obstruction. Comorbidities are highly prevalent in COPD, regardless of its severity. Certain risk factors for CVD, themselves classified as diseases (including smoking, dyslipidemia, and depression), appear to be more prevalent in patients with mild-to-moderate COPD. [13,14,15]. In COPD, comorbid diseases increase the risk of hospitalization and death. Also, comorbidity significantly increases the cost of medical care for such patients. Many of patients with COPD, concomitant pathology worsens the quality of their life and survival, reduces their activity. The presence of concomitant pathology can lead to late diagnosis of COPD [16]. Previous studies have established that COPD is associated with hypertension, kidney diseases and coronary heart diseases (CHD), which this study confirmed. CHD is frequently underdiagnosed in people with COPD; this is important as the coexistence of CHD and COPD results in a worse prognosis than if a patient has one disease or the other. This study demonstrated that individuals with COPD, compared with people without, were more likely to have heart failure, peripheral vascular disease, and cerebrovascular disease consistent with higher rates of hypertension and CHD, with smoking as a common risk factor. Research by Rutten *et al* identified a prevalence of 20.5% for heart failure in people with COPD — compared with 6.4% in this study — when screened for the condition using a number of diagnostic tests including an echocardiogram. It is therefore likely that the true prevalence of heart failure in COPD is higher than found in this study, because patients with COPD are not routinely screened for heart failure [17]. The early impairment of renal function in patients with COPD and comorbid cardiovascular disease is of interest. Unfortunately, this process is asymptomatic for a long time, but it aggravates the course of pulmonary pathology. This issue is not given due attention, which is reflected in the literature [18].

CONCLUSIONS

The presence of comorbid arterial hypertension leads to the intermittent effect of diseases: according to the results of mMRC and SAT feeding, ailments for COPD were examined, they have a more severe course of underlying seizure in the presence of concomitant arterial hypertension. Clinical manifestations in patients with COPD and AH are more severe compared to clinical manifestations in patients without aggravated diseases of the cardiovascular system. Concomitant arterial hypertension enhances the manifestations of bronchial obstruction, in the same way as with patients with isolated COPD.

REFERENCES

1. Yatsenko O.V. Porivnyal'na kharakterystyka rivnya pokaznykiv matrychnoyi metalloproteinazy-9 i vysokochutlyvoho S-reaktyvnoho bilka pry komorbidnosti perebihu arterial'noyi hipertenziyi ta khronichnoyi obstruktyvnoyi khvoroby lehen' [Comparative characterization of the level of indicators of matrix metalloproteinase-9 and highly sensitive C-reactive protein in the comorbid course of hypertension and chronic obstructive pulmonary disease]. Scientific Journal "Science Rise: Medical Science". 2017;5(13): 32-6 (In Ukrainian).
2. Global Initiative for Chronic Obstructive Lung Diseases (GOLD). Global strategy for diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI. WHO workshop report [Internet]. WHO.-2018.
3. Boiko O., Rodionova V. The effect of nutritional status on the degree of arterial hypertension.Chest.2020;158(4). doi: https://doi.org/10.1016/j.chest.2020.08.155
4. Cavaillès A., Brinchault Rabin G., Dixmier A. et al. Comorbidities of COPD. European Respiratory Review. 2013; 22:454–475.
5. Ho T., Cusack R.P., Chaudhary N. et al. Under- and over-diagnosis of COPD: a global perspective. Breathe (Sheff). 2019;15(1): 24-35 doi: 10.1183/20734735.0346-2018.
6. Yin H., Yin S., Lin Q. et al. Prevalence of comorbidities in chronic obstructive pulmonary disease patients: A meta-analysis. Medicine (Baltimore). 2017;96 (19):e6836.
7. Raherison C., Ouaalaya E.H., Bernady A. et al. Comorbidities and COPD severity in a clinic-based cohort. BMC Pulm Med. 2018;18(1): 117 doi: 10.1186/s12890-018-0684-7.
8. Williams B., Mancia G., Spiering W. et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. European Heart Journal. 2018;39(33): 3021-104.
9. Pertseva T.A., Bialik H. KHOZL: sovremennoye sostoyaniye problemy. [COPD: current state of the problem]. Ukrainian pulmonological journal. 2010; 1: 18-19. (In Russian).

10. Yatsenko O.V. Porivnyal'na kharakterystyka rivnya pokaznykiv matryksnoy metalloproteinazy-9 i vysokochutlyvoho S-reaktyvnoho bilka pry komorbidnosti perebihu arterial'noyi hipertenziyi ta khronichnoyi obstruktyvnoyi khvoroby lehen' [Comparative characterization of the level of indicators of matrix metalloproteinase-9 and highly sensitive C-reactive protein in the comorbid course of hypertension and chronic obstructive pulmonary disease]. Scientific Journal "Science Rise: Medical Science". 2017;5(13): 32-6. (In Ukrainian).
11. Lang T.A., Sesik M. Yak opysuvaty statystyku v medytsyni. Kerivnytstvo dlya avtoriv, redaktoriv i retsenzentiv. [How to describe statistics in medicine. Guide for authors, editors and reviewers]. Practical Medicine. 2011;47-49. (In Russian).
12. Karoli N.A., Rebrov A.P. Arterial hypertension in patients with bronchial asthma and chronic obstructive pulmonary disease. The Clinician.2011;5(2):20-30. (In Russian). https://Doi.Org/10.17650/1818-8338-2011-2-20-30.
13. Aronson S., Dyke C.M., Stierer K.A. et al. The ECLIPSE trials: comparative studies of clevidipine to nitroglycerin, sodium nitroprusside, and nicardipine for acute hypertension treatment in cardiac surgery patients. Anesth Analg. 2008;107(4):1110-21. doi: 10.1213/ane.0b013e31818240db.
14. Caram L.M., Ferrari R., Naves C. R. et al. Risk factors for cardiovascular disease in patients with COPD: mild-to-moderate COPD versus severe-to-very severe COPD. J. Bras. Pneumol. 2016;42 (3):179–184.
15. Rashid ur H. Electrolyte disturbances in acute exacerbation of COPD. J Enam Med Col. 2019;9(1): 25-9.
16. Gashynova E.Yu. Sistemnyye effekty i komorbidnost' u ambulatornykh patsiyentov s KHOZL [Systemic effects and comorbidity in outpatients with COPD]. Ukrainian pulmonological journal. 2013; 2: 41-45. (In Russian).
17. Barnett K., Mercer S.W., Norbury M. et al. Epidemiology of multimorbidity and implications for health care, research, and medical education: a cross-sectional study. Lancet. 2012; 380: 37–43.
18. Morgan A.D., Zakeri R., Quint J.K. Defining the relationship between COPD and CVD: what are the implications for clinical practice? Ther Adv Respir Dis. 2018;12. doi: 10.1177/1753465817750524.

ORCID and contributionship:
Viktoriia V. Rodionova: 0000-0002-0221-5625 [A,B,C]
Olha O. Boiko: 0000-0003-0506-0486 [D,E,F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Rodionova V. Viktoriya
Dnipropetrovsk Medical Academy
of the Ministry of Health of Ukraine
9 V. Vernadskogo st., 49044 Dnipro, Ukraine
tel: +38 (067) 912 82 94
e-mail: dsma@dsma.dp.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

EFFECT OF PROLONGED TREATMENT WITH BIOLOGICAL THERAPY IN PATIENTS WITH ULCERATIVE COLITIS WITH CONCOMITENT JOINT DAMAGE

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Antonina V. Varvarynets[1], Ivan V. Chopey[1], Ksenia I. Chubirko[1], Artur V. Kurakh[1], Vasyl M. Voronych[2], Yuriy P. Skrypynets[2]
[1]STATE HIGHER EDUCATIONAL INSTITUTION "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE
[2]COMMUNAL NON-COMMERCIAL ENTERPRISE "UZHHOROD REGIONAL CLINICAL HOSPITAL OF UZHHOROD REGIONAL COUNCIL OF ZAKARPATTIA REGION", UZHHOROD, UKRAINE

ABSTRACT

The aim: Is to compare the effects of tofacitinib, adalimumab and budesonide clinical and laboratory signs of patients with moderate UC with concomitant articular syndrome.

Materials and methods: 100 patients with moderately severe UC. Patients were divided into 2 groups. The I group consisted of patients with UC, which were treated with tofacitinib 10mg bid (TOF; n=28). II group consisted of patients who were treated with adalimumab with a starting dose of 160 mg and 80 mg on the 2nd week of treatment, after which they received a subsequent dosage of 40 mg per week (ADA; n=32). Patients in the III group received budesonide 9mg qd (BUD; n=40).

Results: As a result of treatment in the TOF research group, leukocyte and CRP levels decreased compared to readings before treatment (from 13.6±2.4*10^9/l and 1698 14.0±1.4 mg/l to 10.6±1.2*10^9/l and 11.0±2.1 mg/l respectively, p<0.05). Hemoglobin levels in this group slightly increased (from 104.2±9.2 g/l to 126.1±10.2 g/l, p<0.05). Among ADA patients, there was also an improvement in laboratory signs: leukocyte and CRP levels decreased (from 13.8±2.8*10^9/l and 16.0±1.2 mg/l to 6.0±2.2*10^9/l and 11.8±1.2 mg/l respectively, r<0.05), hemoglobin increased (from 103.8±8.2 g/l to 118.6±8.6 g/l/l r<0.05).

Conclusions: Tofacitinib and adalimumab in the treatment of patients with ulcerative colitis of moderate severity with concomitant joint damage showed a higher clinical and laboratory effectiveness compared to treatment with budesonide.

KEY WORDS: ulcerative colitis, articular syndrome, tofacitinib, adalimumab

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INTRODUCTION

Ulcerative colitis (UC) – remains one of the most relevant problems of modern gastroenterology since its etiology is not fully understood. It clinically manifests as progressive inflammatory-necrotic lesions of the mucous membrane and submucous layer of the colon (local or diffuse) with the development of varying degrees of hemorrhages and ulcers. Characterized by periods of exacerbations and remission [1].

One of the most common extraintestinal complications of UC is joint damage. Peripheral arthritis occurs in 20-30% of UC patients, sacroiliitis – in 10-15%, ankylosing spondylarthritis – in 3-4%.

The most common form of joint lesion in patients with UC, is peripheral arthritis, which affects the hands, feet, elbows, wrists, knees. Pain can move from one joint to another. The activity of inflammation in the joints usually correlates with the degree of inflammation activity in the colon. Symptoms of peripheral arthritis usually disappear without causing long-term damage when the symptoms of UC are properly managed [2,3].

Axial arthritis – is a form of arthritis also known as spondylitis or spondylarthritis, accompanied by pain in the lower back and sacroiliac joints. Notable is the fact, that pain can precede the appearance of intestinal symptoms of UC.

Sacroiliitis in UC is predominantly bilateral. Roentgenologic manifestations appear earlier than symptomatic manifestations of UC and have a different degree of severity, up to the ankylosis of sacroiliac joints.

Ankylosing spondylitis is a more severe form of arthritis. It usually develops in people with a genetic predisposition to bowel or urinary tract infections and tends to manifest before the age of 30.

Diagnosis ofarthropathy in UC is carried out taking into account the following factors: the correlation between articular and intestinal manifestations; parallel occurrence of exacerbation of intestinal manifestations of UC and articular syndrome, predominant damage to large joints, asymmetry of articular syndrome; short-term articular manifestations; development of spondylitis and symmetrical sacroiliitis, especially in the carrier of the HLA-B27 gene; presence of X-ray signs. Laboratory markers of inflammation are also taken into account [4,5].

Management of patients with UC and articular syndrome is based on the effective treatment of the underlying disease. The drugs of choice are 5-amino acid group medications (mesalasine, mesacol, salofalk, etc.), which affect both intestinal

Table I. Dynamics of indicatorsof complete blood count during treatment.

	Study group (TOF, n=28)		Study group (ADA, n=32)		Study group (BUD, n=40)	
Indicator	1 week	52 weeks	1 week	52 weeks	1 week	52 weeks
Red blood cells, 10^{*9}/l	3,42±0,44	4,72±0,22	3,60±0,42	4,64±0,22	3,56±0,42	4,42±0,14
Leukocytes, 10^{*9}/l	13,60±2,42*#	5,82±2,04	13,82±2,81*#	6,04±2,22	14,02±2,34*#	7,24±1,81
Hemoglobin, g / l	104,22±9,20*#	126,14±10,24*#	103,82±8,24*#	118,60±8,62*#	104,64±9,94*#	116,24±7,12*#
Hematocrit	0,41±0,03	0,43±0,02	0,42±0,01	0,43±0,03	0,43±0,1	0,41±0,1
Platelets, 10^9/l	488,0±11,42	327,12±10,10	468,02±10,44	386,04±12,62	456,08±11,82	398,02±11,04
Lymphocytes, %	15,82±1,30	24,04±1,72	16,22±1,34	23,80±1,72	16,64±1,32	24,20±1,20
Neutrophils, %	68,64±4,22	62,82±4,06	64,62±4,02	62,02±4,24	62,44±4,04	60,34±4,10
Monocytes, %	4,20±0,14	9,20±0,22	4,06±0,12	4,82±0,12	4,74±0,10	3,92±0,34
Eosinophils, %	0,60±0,10	3,72±0,90	0,64±0,22	2,64±0,20	0,82±0,14	1,12±0,12
Basophils, %	0,32±0,04	0,62±0,01	0,32±0,04	0,34±0,04	0,64±0,12	0,82±0,12
Neutrophils abs., 10^9/l	9,60±0,90*#	4,82±0,64	9,24±0,40*#	5,24±0,62	8,88±0,22*#	5,24±0,11
Lymphocytes abs. 10^9/l	1,12±0,14	2,44±0,92	1,14±0,24	1,86±0,12	1,24±0,20	1,14±0,12
Monocytes abs., 10^9/l	0,86±0,08	0,84±0,08	0,86±0,06	0,84±0,02	0,88±0,02	0,84±0,02
Eosinophils abs,.10^9/l	0,24±0,02	0,18±0,04	0,24±0,02	0,24±0,02	0,32±0,08	0,12±0,04
Basophiles abs., 10^9/l	0,04±0,01	0,02±0,01	0,04±0,02	0,04±0,02	0,04±0,02	0,02±0,01
Reticulocytes, %	1,82±0,90	1,62±0,22	1,94±0,44	1,64±0,92	1,94±0,12	1,76±0,42
CRP, mg/l	14,0±1,4*#	11,0±2,1*#	16,0±1,2*#	11,8±1,2*#	14,2±0,2	12,2±0,9

* - the difference is statistically probable when comparing indicators before and after treatment, $p<0.05$.
# - the difference is statistically probable when compared to the control group, $p<0.05$.

Table II. VAS index before and after treatment.

VAS index cm	TOF	ADA	BUD
Before treatment	8.6±0.2 cm*#	8.5±0.2 cm*#	8.4±0.3 cm*#
52 weeks	8.6±0.2 cm *#	8.5±0.2 cm *#	8.6±0.2 cm *#

* - the difference is statistically probable when comparing indicators before and after treatment, $p<0.05$.
# - the difference is statistically probable when compared to the control group, $p<0.05$.

and articular symptoms. In case of significant refractoriness to these drugs, glucocorticoids are prescribed additionally [6].

In severe forms of UC, immunosuppressants (cyclosporin, azathioprin and metottrexate) are also used.

In recent years, the number of severe forms of UC has increased dramatically, and anti-TNF drugs (adalimumab) and Janus-kinase inhibitors (tofacitinib) have been added to treatment regimens [7,8].

THE AIM

The aim of the study is to compare the effects of tofacitinib, adalimumab and budesonide on clinical and laboratory signs of patients with moderate UC with concomitant articular syndrome.

MATERIALS AND METHODS

The study was conducted in Uzhhorod Regional Clinical Hospital of Uzhhorod Regional Council of the Transcarpathian Regionfrom 2017 to 2018. 100 patients with moderately severe UC aged between 18 and 75 years old were included in the study. The average age of patients was 39.2±12.6. At the time of inclusion in the study, the disease duration was not less than 6 months. The activity of the disease was determined by the clinical and endoscopic activity index. Patients were divided into 2 groups. The I group consisted of patients with UC, which were treated with tofacitinib 10mg bid (TOF; n=28). II group consisted of patients who were treated with adalimumab with a starting dose of 160 mg and 80 mg on the 2nd week of treatment, after which they received a subsequent dosage of 40 mg per week (ADA; n=32). Patients in the III group received budesonide 9mg qd (BUD; n=40).

Examination of patients with complaints of joint pain included: physical examination conducted by counting swollen and painful joints. The intensity of arthralgia was determined using the visual-analog scale (VS), which is a line of 10 cm long, where the mark 0 corresponds to the value of "pain is absent", 10 cm – "maximum pain". To assess the severity of pain, the patient is offered to put a mark on the scale, which corresponds to the intensity of pain.

Also, laboratory signs were evaluated during treatment.

RESULTS

The study lasted 52 weeks. At the beginning of the study, all patients had a slight decrease in hemoglobin levels, which can be explained by the presence of chronic bleeding. There was also an increase in leukocyte levels and CRP in the blood, which indicates the presence of an inflammatory process.

When evaluating the clinical response in all groups at the end of treatment, in the TOF research group, leukocyte and CRP indicators decreased compared to similar indicators before treatment (from 13.6±2.4* 10^9 /l and 14.0±1.4 mg/l to 10.6±1.2* 10^9 /L and 11.0±2.1 mg/l respectively, r<0.05). Hemoglobin levels in this group slightly increased (from 104.2±9.2 g/l to 126.1±10.2 g/l, p<0.05). Among ADA patients, there was also an improvement in laboratory signs: leukocyte and CRP levels decreased (from 13.8±2.8*10^9/l and 16.0±1.2 mg/l to 6.0±2.2*10^9/l and 11.8±1.2 mg/l respectively, r<0.05), hemoglobin increased (from 103.8±8.2 g/l to 118.6±8.6 g/l/l r<0.05).

In patients receiving budesonide, statistically significant changes in laboratory signs were not observed. (Table I).

Joint lesions were seen in a significant number of patients in all groups. In the TOF group, 11 (39,2 %) patients had arthropathies, which included peripheral arthritis – 6 (21,4%), sacroiliitis – 3(10,7%), AS – 2 (7,14%) patients. In the ADA group, joint damage was observed in 12 (37.5%) patients, including peripheral arthritis – in 6 (18.75%), sacroiliitis – in 4 (12.5%), AC – in 2 (6.25%) patients. In the BUD group, joint damage was noted in 15 (37.5%) patients, including peripheral arthritis – 7 (17.5%), sacroiliitis – in 5 (12.5%), AC – 3 (7.5%) patients. At the beginning of treatment, there was no statistically significant difference in the degree of pain between all of the studied groups (8.6±0.2cm, 8.5±0.2cm and 8.4±0.3cm on the VAS scale in the TOF, ADA and BUD groups, respectively, p>0.05). (Table II).

DISCUSSION

In this study, we investigated the changes in clinical and laboratory signs of patients with moderately severe UC when treated with budesonide, adalimumab and tofacitinib. Gathered data enables us to better understand the course of the disease and to monitor the safety of management of ulcerative colitis with these drugs.

When comparing the study indicators between patients of the study and control groups, it was found that in the tofacitinib and adalimumab group, the leukocyte values and CRP were statistically lower, and hemoglobin was higher, compared to the budesonide group. These findings correlate with results of previous studies, which were considered during UC management planning [8,9,10].

After treatment in the TOF research group, the VAS index decreased compared to the similar indicator before treatment (from 8.6±0.2 cm to 5.6±0.3 cm, p<0.05). In the ADA group, a decrease in this indicator compared to the same treatment was also observed (from 8.5±0.2 cm to 6.2±0.2 cm, p<0.05). In the BUD group there were no statistically probable changes in the degree of pain. At the same time, the severity of pain on the VAS scale after treatment between patients in the group of tofacitinib and adalimumab was statistically lower compared to same values in the budesonide group.Similar results were seen in other published research [11,12].

Tofacitinib and adalimumab provide a better therapeutic effect in patients with moderate ulcerative colitis and articular syndrome, when compared to budesonide. Further research is needed to study the financial aspects of patient management with drugs that were mentioned above.

CONCLUSIONS

Tofacitinib and adalimumab in the treatment of patients with ulcerative colitis of moderate severity with concomitant joint damage showed a higher clinical and laboratory effectiveness compared to treatment with budesonide. Further research is needed, in particular on the study of pharmaco-economic aspects of the use of these drugs.

REFERENCES

1. Feuerstein J., Cheifetz A. Ulcerative colitis: epidemiology, diagnosis, and management MayoClin Proc. 2014; 89(11):1553-1563.
2. Vavricka S., Rogler G., Gantenbein C. et al. Chronological Order of Appearance of Extraintestinal Manifestations Relative tothe Time of IBD Diagnosis in the Swiss Inflammatory Bowel Disease Cohort.Inflamm Bowel Dis. 2015;21(8):1794-1800.
3. Harbord M., Annesse V., Vavricka S. et al .The First European Evidence-based Consensus on Extra-intestinal Manifestationsin Inflammatory Bowel Diseas. Journal of Crohn's and Colitis. 2015;10(3):239-254.
4. Sieper J. Poddubnyy D. Axial spondyloarthritis.Lancet. 2017:390 (10089);73-84.
5. Pouillon L., Bossuyt P., Vanderstukken J. et al. Management of patients with inflammatory bowel disease and spondyloarthritis. Expert Review Of Clinical Pharmacology. 2017:10(12);1363-1374.
6. Panés J., Vermeire S., Lindsay J. et al. Crohns Colitis. Tofacitinib in Patients with Ulcerative Colitis: Health-Related Quality of Life in Phase 3 Randomised Controlled Induction and Maintenance Studies.2018;24;12(2):145-156.
7. Izzo R., Bevivino G., Monteleone G. Tofacitinib for the treatment of ulcerative colitis.Expert OpinInvestig Drugs. 2016;25(8):991-7. doi: 10.1080/13543784.2016.1189900.
8. Izzo R., Bevivino G., Monteleone G. Tofacitinib for the treatment of ulcerative colitis.Expert OpinInvestig Drugs. 2016;25(8):991-7. doi: 10.1080/13543784.2016.1189900.
9. Taxonera C., Iglesias E., Munoz F. et al. Adalimumab maintrance treatment in ulcerative colitis: outcomes by prior anti-TNF use and efficacy of dose escalation. Dig Dis Sci. 2017;62:481-90.
10. Balint A., Farkas K., Palatka K. et al. Efficacy and safety of adalimumab in ulcerative colitis refractory to conventional therapy in routine clinical practice. J Crohns Colitis. 2016;10:26-30.
11. Botteman M.F., Hay J.W., Luo M.P. et al. Cost effectiveness of adalimumab for the treatment of ankylosing sponditis in the United Kingdom. Rheumatology. 2007;46:1320-1328.
12. Van der Heijde D., Deodnar A., Wei J.C. et al. Tofacitinib in patients with ankylosing spondylitis: a phase 2, 16-week, randomized, placebo-controlled, dose-ranging study. Ann Rheum Dis. 2017. doi:10.1136 annrheumdis-20160210322.

ORCID and contributionship:
Antonina V. Varvarynets: 0000-0001-5859-1040 [A,D F]
Ivan V. Chopey: 0000-0003-4626-0855 [A,F]
Ksenija I. Chubirko: 0000-0002-4379-0538 [A,B]
Artur V. Kurakh: 0000-0003-2793-5044 [E,C]
Vasyl M. Voronych: 0000-0001-9718-3496 [A,B]
Yuriy P. Skrypynets: 0000 0002 9741 4175 [B,C]

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CORRESPONDING AUTHOR
Antonina V. Varvarynets
Uzhhorod national university
71 Minayska St., 88000 Uzhhorod, Ukraine
tel: +380679810601
e-mail: tonichka8387@gmail.com

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ORIGINAL ARTICLE

PECULIARITIES OF DETERMINING THE GASTRIC ACID SECRETION AND DIABETIC AUTONOMIC NEUROPATHY IN PATIENTS WITH CHRONIC PANCREATITIS AND TYPE 2 DIABETES

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Yelyzaveta S. Sirchak, Vasilij Ye. Barani, Olena M. Odoshevska, Oksana I. Petrichko
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: Is to determine the peculiarities of changes in the gastric acid secretion against the background of diabetic autonomic neuropathy and autonomic dysfunction in patients with chronic pancreatitis (CP) and type 2 diabetes (T2DM).

Materials and methods: We investigated 64 patients with CP and T2DM, who were included in the first group of the patients examined; Group II consisted of 40 patients with T2DM; and Group III of the patients examined consisted of 34 patients with CP.

Results: Differences were found in assessing the degree of autonomic nervous system (ANS) dysfunction in the examined patients, namely – the most pronounced ANS dysfunction according to the Wayne questionnaire was diagnosed in patients Group I, while patients of Group III. Analysis of gastric acid secretion indicates that no patients of Group III had normal acidity. Normal acidity is more often found in the second group of subjects. Both in patients with CP and T2DM, and in isolation with CP, moderate hyperacidity was more often determined.

Conclusions: The predominance of the parasympathetic division of the ANS, as well as manifestations of severe ANS dysfunction, are observed in patients with CP and T2DM. The prevalence of gastric hyperacidity on the background of DAN was established in patients with CP and T2DM. In this case, the absence of clinical symptoms or their minimal severity is determined, which indicates the lesion of the digestive tract in these patients.

KEY WORDS: chronic pancreatitis, type 2 diabetes mellitus, diabetic autonomic neuropathy, autonomic dysfunction, gastric acid secretion of the stomach

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INTRODUCTION

Diabetes mellitus (DM) is a specific disease, on the background of which there has been an increase in comorbid pathologies in the recent years. Diabetes accounts for disruption of all types of substances of metabolism and affects all organs and systems of the body including the digestive system (esophagus, stomach, intestine, liver and pancreas) [1]. Of particular interest is the study of the combined course of chronic pancreatitis (CP) and diabetes (DM) simultaneously affecting two different functions of the same organ [2, 3].

Chronic pancreatitis (CP) is one of the most complex polyethiological and polymorbid diseases [4]. The summation and potentiation of adverse effects of risk factors and the action of etiological factor of CP contribute to the growth of its incidence rate and progressive course, with exocrine insufficiency of the pancreas, development of maldigestion, malabsorption and trophological insufficiency in patients with impaired metabolism of all substances [5, 6].

Failure of the autonomic nervous system, i.e. diabetic autonomic neuropathy (DAN) is a serious and common complication that is observed in 16.8–54.0% of DM patients. However, DAN remains among the least studied complications due to its long asymptomatic course. It is often diagnosed late, and therefore difficult to treat due insufficiently developed methods of early diagnosis and treatment. Diabetic autonomic gastrointestinal neuropathy, with its characteristic gastroparesis, enteropathy and cholecystoparesis, is one of the main causes of labile course of the disease. It also increases the risk of gallstone disease 2-5 times in patients with DM compared to the general population [7].

Given the above facts, detecting DAN in patients with type 2 diabetes, especially when combined with CP, is of important diagnostic value for these patients. In this process, the study of the functional state of the upper abdominal organs, namely the biliary system, gastroesophageal area (with the focus on the gastric acid secretion can largely serve as one of the main elements for early diagnosis of digestive changes that can aggravate the clinical course of the existing pathology.

THE AIM

Aim of the research is to determine the peculiarities of changes in the gastric acid secretion against the background of diabetic autonomic neuropathy and autonomic dysfunction in patients with CP and type 2 diabetes.

Table I. Evaluation of DAN indicators in the examined patients and the control group

Indicator	Control group (n=20)	Examined patients		
		Group I (n=64)	Group II (n=40)	Group III (n=34)
Ewing test, beats/min	21.71±3.15	5.28±0.67*, ++,^	8.67±0.88 *,++	20.35±1.23
Shellong test, mmHg	6.78±1.45	52.13±7.11 *, ++,^	40.71±4.22 *,++	7.88±1.25
Test 30:15	2.38±0.27	0.68±0.17 *, ++	0.92±0.15 *,+	1.89±0.12

Note: between the indicators of the control group and the examined patients of Groups I, II and III the difference is statistically significant: * - $p < 0.01$; the difference between the indicators in patients of Group III and patients of Groups I and II is statistically significant: + - $p < 0.05$; ++ - $p < 0.01$; the difference between the indicators in patients of Group I and Group II is statistically significant: ^ - $p < 0.05$.

MATERIALS AND METHODS

64 patients with CP and type 2 diabetes were examined and divided into the first group of the patients (35 male (54.7 %) and 29 female (45.3 %) of an average age of 48.9 ± 6.3). Group II consisted of 40 patients with type 2 diabetes (22 men (55.0%) and 18 women (45.0%) at an average age of 48.9 ± 6.3 years); and Group III of the patients consisted of 34 patients with CP (21 male (61.8%) and 13 female (38.2%) of an average age of 46.7 ± 4.2 years). All examined patients were either hospitalized in the Endocrinology and Gastroenterology Departments of Municipal Non Profit Enterprise "Transcarpathian Regional Clinical Hospital Named After Andrii Novak" of Transcarpathian Regional Council or were on outpatient observation by the district family doctors at the place of their residence.

The control group included 20 healthy individuals (11 male (55.0%) and 9 female (45.0%) of an average age of 45.2 ± 5.1).

All studies were performed with the consent of the patients, and their methodology was in line with the Helsinki Declaration of Human Rights of 1975 and its revision of 1983, the Council of Europe Convention on Human Rights and Biomedicine and the current legislation of Ukraine.

All patients were examined using general clinical, anthropometric, instrumental and laboratory research methods. All patients also underwent ultrasound examination of the abdominal cavity by conventional methods. Standard general and biochemical studies of blood serum were conducted with an emphasis on carbohydrate metabolism (glucose, glycated hemoglobin, insulin, C-peptide, proinsulin and glucose tolerance test).

The diagnosis of CP was established in accordance with the Marseille-Roman criteria (1989) with the addition of Ya.S. Zimmerman (1995) and clarifications of ICD-10. A coprological study was performed, serum amylase and fecal elastase-1 levels were determined, and ^{13}C-mixed triglyceride (^{13}C-CTDT) and ^{13}C-amylase respiratory tests (^{13}C-ADT) were used to study the exocrine function of the pancreas,

Medical care was provided to the examined patients with diabetes according to the clinical protocols of the Ministry of Health of Ukraine and local protocols. The diagnosis of type 2 diabetes was established in accordance with the recommendations of the IDF (2005), as well as taking into account the criteria of a unified clinical protocol (The Ministry of Health of Ukraine Order of 21.12.2012 № 1118) [8, 9].The severity of type 2 diabetes was assessed by the level of HbA1c (Normal: up to 6.0%).

To diagnose diabetic autonomic neuropathy (DAN), all patients underwent a cardiac rhythmogram (with Polar Pro heart rate monitor), during which heart rate was measured using a sensor that was applied to the chest over the projection of the apex of the heart, with the recording of heart contractions. Assessment of cardiovascular diabetic neuropathy was performed by standard Ewing tests (assessment of changes in heart rate (HR) with slow deep breathing (6 breaths per minute)); Shellong test (orthostatic test) and 30:15 test, as these tests are proposed as a standard for the diagnosis of DAN (San-Antonio, 1998).

Detection of autonomic dysfunction (AD) was performed using the O.M. Wayne's questionnaire (1998). The score above 15 indicated AD. Also, the Kérdö's Vegetative Index was calculated (KVI) on the basis of hemodynamic parameters (HR, blood pressure):

KVI = (1-diastolic blood pressure (DBP) / pulse) * 100.

At full vegetative balance (eytonia) the index is in the range from -10 to +10. With the predominance of sympathetic influences (sympathicotonia) the value of KVI will be higher than +10, and with the predominance of parasympathetic influences (vagotonia) the value of KVI will be lower than -10.

To study the gastric acid secretion of the stomach, patients underwent intragastric express pH-metry, using a computer system according to the method of Prof. V.N. Chernobrovy. The study was conducted in the morning (7.00-9.00 hours). To accurately assess basal acidity 24 hours before the study, the use of any antacids was excluded.

Each patient was administered a pH microprobe through the mouth, the beginning of the pH measurement was carried out at the level of the transition of the esophagus into the stomach, which in most patients was 40 cm at the depth of the probe (from incisors). For the entire length of the stomach (from the cardia to the pylorus), the pH was measured every 1 cm, each time for 15-20 seconds. The technique of basal topographic pH-metry requires both the introduction and removal of the pH microprobe along the entire length of the stomach. There was a time interval of 5-10 minutes between the input and output of the probe. To evaluate the results, we took into account the functional pH interval/range (FI pH) from 0 to 5 (in the direction of increasing gastric acidity):

Table II. Indicators of the functional state of the ANS in the examined patients and the control group

Indicator	Control group (n=20)	Examined patients		
		Group I (CP+T2DM) (n=64)	Group II (T2DM) (n=34)	Group III (CP) (n=40)
Vegetative state according to O.M.Wayne scale, points	9.82±0.23	55.71±3.23**,+	43.17±5.30**,+	28.74±2.50*
HR per minute	70.40±3.82	98.17±4.35*	89.56±4.89*	87.11±4.23*
SBP, mm Hg	114.15±7.92	158.26±5.54*	147.80±6.11	130.42±5.34
DBP, mm Hg	74.72±5.81	109.71±5.34*	98.11±3.90*	77.17±5.32
KVI	-6.55±-0.44	-13.70±1.89*,+,^	-10.78±1.25*,+,^	10.88±2.40*

Note: between the indicators of the control group and the examined patients of Groups I, II and III the difference is statistically significant: * - p <0,05; ** - p <0.01; the difference between the indicators in patients of Group III and patients of Groups I and II is statistically significant: + - p <0,05; ++ - p <0.01; the difference between the indicators in patients of Group I and Group II is statistically significant: ^ - p <0,05.

pH 7,0 – 7,5 (FI pH0 – anacidity);
pH 3,6 – 6,9 (FI pH1 – pronounced hypoacidity);
pH 2,3 – 3,5 (FI pH2 – moderate hypoacidity);
pH 1,6 – 2,2 (FI pH3 – normal acidity);
pH 1,3 – 1,5 (FI pH4 – moderate hyperacidity);
pH 0,9 – 1,2 (FI pH5 – pronounced hyperacidity).

According to the method of basal topographic pH-metry, it is important to characterize the distribution of FI pH (in percent) over the entire length of the stomach, which included the individual functional maximum acidity of the stomach. On this basis, gastric acidity is divided into:

- minimal (up to 25% of the total number of pH measurements);
- selective (26-50% of the total number of pH measurements);
- absolute (51-75% of the total number of pH measurements);
- subtotal (76-99% of the total number of pH measurements);
- total (100% of pH measurements).

Criteria for exclusion of patients from the study were: type 1 diabetes, type 2 diabetes (severe with severe manifestations of diabetic angioneuropathy), active gastric and duodenal ulcers, erosive gastroduodenitis, gastroesophageal reflux disease, and the presence of Helicobacter pylori.

The analysis and processing of the results of the examination was carried out by means of the STATISTICA 10.0 computer program (StatSoft Inc., USA) using parametric and nonparametric methods for evaluating the results.

RESULTS

In all examined patients, the determination of DAN was performed according to the above methods. The results are presented in Table 1.

Evaluating the obtained data, DAN was detected in patients of Group I (patients with CP and type 2 diabetes) and Group II (patients with type 2 diabetes), as evidenced by a decrease in heart rate with slow deep breathing, which confirms the violation of parasympathetic innervation of the heart. Thus, this indicator was significantly lower in patients of Groups I and II, with a significant difference between these surveyed groups, i.e. worse indicators were observed in patients with CP and type 2 diabetes (5.28 ± 0.67 beats / min) than in patients of Group II (type 2 diabetes without CP) -8.67 ± 0.88 beats / min, respectively, (p <0.05). The result of this test was within normal limits in patients with CP and did not differ significantly from the control group. As for the results of the orthostatic test, this figure was increased in patients of Groups I and II. In addition, there was a significant difference between this indicator in patients with CP and those without CP – and amounted to 52.13 ± 7.11 mm Hg and 40.71 ± 4.22 mm Hg. correspondingly. This may indicate a violation of sympathetic innervation and, accordingly, DAN. Another diagnostic criterion for DAN is the 30:15 test. The indicators of this test were also significantly higher in patients of Groups I and II, while in patients with CP (group III), the indicators did not differ significantly from the control group.

The analysis of research on the definition of ANS dysfunction indicates its presence in the examined patients also mainly I and II groups according to the results of the questionnaire (O.M. Wayne questionnaire) and KVI. Indicators of the functional state of the autonomic nervous system (ANS) in the examined patients are presented in table 2.

The analysis of the obtained clinical data indicates that the majority of examined patients of Groups I and II complained of frequent emotional stress, mood lability, increased irritability, anxiety, sleep disturbances, headache, fatigue, heart palpitations, and heart failure due to negative emotions. In addition, differences were found in assessing the degree of ANS dysfunction in the examined patients, namely – the most pronounced ANS dysfunction according to the Wayne questionnaire was diagnosed in patients with CP in combination with type 2 diabetes (group I) – 55.71 ± 3.23 points, while patients of Group III (patients with CP) had the lowest values (28.74 ± 2.50 points) on this scale.

Further analysis of the indicators of the functional state of the ANS indicates the predominance of the influence of its parasympathetic division in patients of Groups I and II. It should be noted that according to the results of KVI statistically significant difference between the examined patients of Groups I and II was not established. In patients

Table III. Characteristics of gastric acidity in the examined patients

Indicator	Examined patients, %		
	Group 1(CP+T2DM) (n=64)	Group 2 (T2DM) (n=34)	Group 2 (CP) (n=40)
Moderate hypoacidity:	**4.7 % ***	**11.7 %**	_
selective	33.3 % **	_	_
absolute	66.7 % *	50.0 %	_
subtotal	_	50.0 % **	_
Normal acidity:	21.9 % *	35.3 %	25.0 %
selective	21.4 %	41.7 %	10.0 %
absolute	35.7 % +	33.3 %	50.0 %
subtotal	42.9 %	25.0 %	40.0 %
Moderate hyperacidity:	42.2 %	32.4 %	45.0% *
minimal	3.7 %	18.2 %	_
selective	7.4 %	36.4 %	27.8 % ++
absolute	22.2 %	27.2 %	33.3 % *
subtotal	29.7 % ++	18.2 %	16.7 %
total	37.0 % **, +	_	22.2 % **
Pronounced hyperacidity:	31.2 % *	20.6 %	30.0 %
minimal	_	42.8 %	_
selective	20.0 %	28.6 %	25.0 %
absolute	25.0 %	14.3 %	25.0 %
subtotal	30. 0 % *	14.3 %	33.3 % *
total	25. 0 % **	_	16.7 % *

Note: the difference between the indicators in patients of Group II and patients of Groups I and III is statistically significant: * - p <0,05; ** - p <0.01; the difference between the indicators in patients of Group I and Group III is statistically significant: ^ - p <0,05.

of Group III (patients with CP) indicators of the functional state of the ANS indicates a slight predominance of the sympathetic division.

Analysis of gastric acid secretion indicates that no patients of Group III had normal acidity. Normal acidity is more often found in the second group of subjects (with type 2 diabetes). Both in patients with CP and type 2 diabetes, and in isolation with CP, moderate hyperacidity was more often determined (in 42.2% of patients of I and 45.0% of patients of Group III). At the same time, in patients of Group I moderate hyperacidity was mostly total and subtotal, while in patients of Group III – more often selective and absolute. Severe hyperacidity is also more common in CP and in patients with a combination of CP and type 2 diabetes. In patients of Group I, pronounced hyperacidity was more often subtotal, total and absolute, and in patients of Group III – total, absolute and selective hyperacidity were more often diagnosed. Patients of Group II (patients with type 2 diabetes) are more likely to have normal acidity and moderate hyperacidity.

It should be noted that in characterizing the clinical manifestations of gastric dyspepsia, such signs as heartburn, acid regurgitation and epigastric pain occurred only among the examined Group III. In patients of the Groups I and II signs of biliary dyspepsia (bitter taste in the mouth, belching with bitter aftertaste), and also heaviness mainly in the right upper quadrant came to the fore.

DISCUSSION

Diabetic autonomic neuropathy (DAN) is a serious and common complication of diabetes, often overlooked and misdiagnosed. Diabetic autonomic neuropathy may involve the cardiovascular, genitourinary, and the neuroendocrine systems as well as the upper and lower gastrointestinal (GI) tract. Abnormalities of GI function in diabetics are thought to be related, at least in part, to autonomic neuropathy [10].

Our studies indicate that in patients with type 2 diabetes, especially in combination with CP, there is an impairment of gastric acid secretion, which is mainly manifested by hyperacidity. However, in these patients symptoms of gastrointestinal lesions are not pronounced against the background of DAN and autonomic disorders, and this in many respects complicates timely diagnosis and adequate pathognetically substantiated treatment.

The professional literature discusses the violation of the acid-forming function of the stomach, mainly in the defeat of the upper gastrointestinal tract and in its combination with other pathological conditions. Skubytska LD, Severynovska OV (2015) indicate the dependence of

the values of gastric juice acidity on the digestive function of the alimentary tract in the diseased with several pathologies – chronic gastritis and with accompanying pancreas affection – has been investigated. The decrease of acid production of the stomach in the diseased I (chronic gastritis) and the diseased II (chronic gastritis with pancreas affection) has been detected, with more noticeable effect in the group with accompanying pathology [11].

Therefore, further study of gastric acid secretion, as well as the influence of DAN on its production against the background of autonomic disorders in patients with CP and type 2 diabetes has important diagnostic value in these patients for the selection of adequate treatment. Further research should also be conducted in this direction to reveal the relationship between DAN and digestive tract lesions, including CP in patients with type 2 diabetes.

CONCLUSIONS

1. Ewing, Shellong and 30:15 tests are effective methods for detecting DAN in patients with CP and type 2 diabetes.
2. The predominance of the parasympathetic division of the ANS, as well as manifestations of severe ANS dysfunction, are observed in patients with CP and type 2 diabetes.
3. The prevalence of gastric hyperacidity on the background of DAN was established in patients with CP and type 2 diabetes. In this case, the absence of clinical symptoms or their minimal severity is determined, which indicates the lesion of the digestive tract in these patients.

REFERENCES

1. Fadejenko G.D., Chernishov V.A. Defeat of the gastroduodenal area in patients with diabetes mellitus: clinical and population aspects. Medicine of Ukraine. 2011; 7(153): 48-50. (in Ukranian).
2. Larin A.S., Tkach S.M. Patogenetocheskaja rol kisechnogodisbioza v razvitii ozhirenia, insulinorezistentnosti I sacharnogo diabeta 2 tipa [Pathogenetic role of kissechnydysbiosis in the development of obesity, insulin resistance and type 2 diabetes mellitus]. Health of Ukraine. Thematic number. Gastroenterology. Hepatology. Coloproctology. 2016; 2(40): 20-21. (in Russian).
3. Babinec L.S., Zacharchuk U.M., Pidruchna S.R. Zastosuvanja balnoji sistemi M-ANNHEIM pri chronichnomu pancreatiti u komorbidnosti z cukrovim diabetom v ambulatornij practici [The use of the M-ANNHEIM score system for chronic pancreatitis in comorbidity with diabetes mellitus in outpatient practice]. Thematic number. Gastroenterology. Hepatology. Coloproctology. 2016; 2(40): 54-55. (in Ukrainian).
4. Babinec L.S., Palihata M.V., Sasik G.M. Possibilities of complex rehabilitation of patients with chronic pancreatitis at the stage of primary care (literaturereview). Pancreatology Club Bulletin. 2018: 4-11.(in Ukrainian).
5. Raksha N.G., Halenova T.I., Vovk T.B. et al. Proteolitic imbalance as a key factor of the development of chronic pancreatitis with and without type 1 diabetes mellitus. Visnik problem biologiii medicine. 2019; 3 (152): 186-191. (in Ukrainian) DOI 10.29254/2077-4214-2019-3-152-186-191
6. Hristich T.M., Hontsariuk D.O. Etiological factors which from the chronical pancreatitis. Zdobutkiklinichnoji I eksperimentalnoji medicine. 2018;3:20-27. (in Ukrainian). doi: 10.11603/1811-2471.2018.v0.i3.9221.
7. Skrypnyk N.V., Gryb V.A., Didushko O.M. Features of the Pathogenesis and Treatment of Diabetic Autonomic Neuropathy (Literature Review). Medicine of Ukraine. 2012; 2 (158): 6-14. (in Ukrainian).
8. Hobzej M.K., Guljchij M.V., Manyjkovskij B.M. Type 1 Diabetes Mellitus in joung people and adults. Unified clinical protocol for primary, emergency, secondary (specialized) and third (highly specialized) medical care. Kyiv; 2014, 71 p. (in Ukrainian).
9. Hobzej M.K., Guljchij M.V., Stepanenko A.V. Type 2 Diabetes Mellitus. Unified clinical protocol for primary and secondary (specialized) medical care. Kyiv; 2012, 118p. (in Ukrainian).
10. Verrotti A., Prezioso G., Scattoni R., Chiarelli F. Autonomic Neuropathy in Diabetes Mellitus. Front. Endocrinol. (Lausanne). 2014; 5: 205.
11. Skubytska L.D., Severynovska O.V. Acid-marking function of stomach with chronic gastritis and chronic gastritis with accompanying pancreas affection. Word of Medicine and Biology. 2014; 4 (54): 69-73.

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ORCID and contributionship:
Yelyzaveta S. Sirchak: 0000-0001-6738-0843 [A,B,C,D]
Vasilij Ye. Barani: 0000-0002-2616-2230 [C,D]
Olena M. Odoshevska: 0000-0003-3412-3554 [E,F]
Oksana I. Petrichko: 0000-0001-5420-6424 [E]

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CORRESPONDING AUTHOR
Yelyzaveta S. Sirchak
Uzhhorod national university
3 Narodna sqr., 88000 Uzhhorod, Ukraine
tel: +380509761794
e-mail: sirchakliza777@gmail.com

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ORIGINAL ARTICLE

INFLUENCE OF COMPLEX TREATMENT ON BIOCHEMICAL BLOOD PARAMETERS OF PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE AND CONCOMITANT PRE-DIABETES

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Snizhana V. Feysa, Svitlana O. Rudakova
STATE HIGHER EDUCATIONAL ESTABLISHMENT "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE

ABSTRACT

The aim: Of this research is to evaluate laboratory changes in the liver blood tests, carbohydrate and lipid metabolism in NAFLD patients with concomitant pre-diabetes, and to study the feasibility of their complex treatment with the inclusion of omega-3 polyunsaturated fatty acids and essential phospholipids.

Materials and methods: We have examined 55 patients with non-alcoholic fatty liver disease on the background of pre-diabetes aged 40 to 75 years. Modification of lifestyle was recommended to all patients as a basic treatment. In addition, the patients were prescribed essential phospholipids in 2 capsules 3 times a day and omega-3 polyunsaturated fatty acids 1000 mg per day for 28 patients (group 1) or rosuvastatin 10 mg per day for 27 persons (group 2). The effectiveness of the treatment was evaluated in 3 months, and the long-term outcomes were evaluated in 12 months.

Results: Under the influence of the prescribed treatment, a hypolipidemic effect was observed in both groups, but a significant decline in the activity of alanine aminotransferase and aspartate aminotransferase occurred only under the influence of a combination of essential phospholipids and omega-3 polyunsaturated fatty acids.

Conclusions: Thus, the described results allow to recommend this combination of medicines to patients with non-alcoholic fatty liver disease and concomitant pre-diabetes.

KEY WORDS: pre-diabetes; dyslipidemia; hepatoprotector; essential phospholipids; omega-3 polyunsaturated fatty acids

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver condition wordwide that begins with excessive accumulation of lipids in hepatocytes, mainly triglycerides (stage of hepatosteatosis) [1], after that it can progress to steatohepatitis [2] and very often predictors (obesity, type 2 diabetes mellitus, dyslipidemia, genetic predisposition) are present with NAFLD [3]. There are controversial views on the appropriateness of the prescribtion of medication in cases where NAFLD is diagnosed in patients with pre-diabetes (PD), characterized by a glucose intolerance or preprandial hyperglycemia, especially if the NAFLD is detected during the initial stage, i.e. hepatosteatosis. A dangerous feature of NAFLD, especially at the stage of steatosis, is the asymptomatic clinical picture, which makes it difficult to timely diagnose and initiate early treatment. Very often, the clinical picture of NAFLD patients is characterized only by the symptoms of concomitant pre-diabetes or type 2 diabetes mellitus (CD-2) (often first detected). Most scientificies tend to recommend lifestyle modification and dietary correction to treat such a comorbid combination. This pathogenetic approach is also well-grounded for hepatosteatosis (contributes to lower lipid content in hepatocytes), and for concomitant pre-diabetes (corrects hyperglycemia) [4]. However, the dyslipidemia that occurs in persons with NAFLD is a predictor for high cardiovascular risk (CVD) [5] and, according to the recommendations of the European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and the European Association for the Study of Obesity (EASO) (2016) requires the prescribing of hypolipidemic pharmacotherapy [6]. Mostly, the statins are used with the purpose of correcting dyslipidemia. However, there is no unambiguousness about their recommending to patients with NAFLD because there is a notice of the probability of side effects due to hepatotoxicity [7]. Therefore, the search is relevant for alternative medicines to statins that would help to correct dyslipidemia, reduce hyperglycemia and have a hepatoprotective effect. Taking into account that NAFLD is a disease characterized by comorbidity, which often leads to polypharmacy [5], we should prefer the multifaceted action medicines for the treatment of such patients [8]. Despite the fact that modern recommendations [6] do not contain instructions about prescribtion of hepatoprotectors, there are papers [9; 10; 11] on the expediency of their using, which is a pathogenetically grounded approach in case of NAFLD. It is proved that the correction of the functional state of hepatocytes contributes to increasing the efficiency of therapy due to indirect effects on the level of insulin resistance (IR) [9].

Table I. Laboratory characteristics of patients with non-alcoholic fatty liver disease and concomitant pre-diabetes (in comparison with patients who have non-alcoholic fatty liver disease with concomitant type 2 diabetes mellitus and in comparison with healthy persons)

Groups, The number of patients in the group (n)	Group 1, NAFLD + pre-diabetes	Group 2, NAFLD + T2-DM	Group 3, Healthy persons
	(n=55)	(n=73)	(n=30)
	Laboratory test, units		
Fasting glucose, mM/l	6,26±0,65 * #	11,1±1,78	4,08±0,59
HbA1C, %	6,04±0,24 * #	7,87±0,52	5,13±0,44
IR-HOMA, U	5,50±2,92	9,34±3,42	2,33±0,23
Total cholesterol, mM/l	5,18±0,56	5,84±0,68	4,03±0,66
Triglycerides, mM/l	2,54±0,41 #	2,77±0,6	1,84±0,26
HDL, mM/l	1,17±0,12 * #	0,906±0,10	2,27±0,76
LDL, mM/l	2,86±0,49	3,67±0,47	2,71±0,34
VLDL, mM/l	1,15±0,19	1,26±0,27	0,83±0,13
Atherogenic index of plasma (AIP), U	3,48±0,73 #	5,48±0,68	2,15±0,43
ALT, IU/l	27,55±6,93 #	37,9±10,52	12,56±3,12
AST, IU/l	22,74±5,17 #	33,8±11,98	8,32±2,89
GGT, IU/l	21,64±14,84	39,3±12,55	10,24±3,61
Alkaline phosphatase (ALP), IU/l	41,33±14,81 *	94,81±14,05	46,57±7,13
Total protein, g/l	70,66±4,74	70,6±3,03	73,86±2,56
Bilirubin total, mkM/l	13,27±3,47	9,74±1,82	10,01±1,24
Bilirubin direct, mkM/l	3,85±0,	3,42±0,9	3,4±0,76
Creatinine, mkM/l	77,54±19,56	77,25±11,51	78,91±8,22
Uric acid, mM/l	277,8±74,77	336,2±53,97	280,21±22,3

Note: * - significant differences with group 2, $p \leq 0.05$; # - significant differences with healthy persons, $p \leq 0.05$

Considering the complex action, cytoprotective, anti-inflammatory, hypotriglyceridemic and antioxidant properties, our attention was attracted by essential phospholipids (EFL) [9; 12] and omega-3 polyunsaturated fatty acids (PUFAs). Building in the biological membrane of hepatocytes, EFL improve membrane-dependent functions, exhibit anti-inflammatory, anti-fibrotic, apoptotic modulating, regenerative, and protective effects [10]. The using of omega-3 PUFAs [13] is justified for the correction of hyperlipidemia, because they are characterized by hypotriglyceridemic action.

The frequent occurrence of NAFLD and the lack of unambiguous recommendations about the use of essential phospholipids and omega-3 PUFAs for their treatment determine the relevance of this work and justify the need to study their clinical efficacy in treatment of such patients.

THE AIM

The aim was to evaluate laboratory changes in the liver blood tests, carbohydrate and lipid metabolism in patients with NAFLD with concomitant pre-diabetes, and to study the feasibility of their complex treatment with the inclusion of omega-3 polyunsaturated fatty acids and essential phospholipids.

MATERIALS AND METHODS

This study is a part of common scientific theme of Therapy and Family Medicine Department "Optimization of prevention and treatment of obesity and diabetes mellitus in cases of Helicobacter pylori associated diseases". The article is also a part of scientific research work for obtaining a Doctor of Medicine degree "Non-alcoholic fatty liver disease and concomitant subclinical hypothyroidism". The study was carried out in accordance with the requirements of the Helsinki Declaration. The Ethical Committee of the Therapy and Family Medicine Department approved the study protocol, and informed consent was obtained from the participants.

We examined 55 patients with NAFLD and pre-diabetes that were treated at the Therapy and Family Medicine Department of Uzhhorod National University in 2011-2017. Their laboratory parameters were compared with similar indices of 73 patients with NAFLD and concomitant type 2 diabetes mellitus (T2-DM) (group 2) and 30 healthy persons, who were involved to the control group (group 3). The groups were standardized by the age and gender.

The NAFLD diagnosis was considered according to the EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease (2016). Inclusion criteria were confirmed diagnoses of NAFLD and pre-diabetes (glucose intolerance or preprandial hy-

Table II. Dynamics of carbohydrate and lipid metabolism indices in patients with NAFLD and concomitant pre-diabetes under the influence of prescribed treatment (M±m)

Blood test	Sub-group 1A (n=28)		Sub-group 1B (n=27)	
	Before treatment	After 3 month treatment	Before treatment	After 3 month treatment
Fasting glucose, mM/l	6,28±0,65	5,99±0,57	6,23±0,66	6,05±0,62
HbA1C, %	6,044±0,25	5,92±0,23	6,036±0,24	5,96±0,23
IR-HOMA, U	4,69±2,39	4,26±1,36	6,32±3,35	6,29±1,81
Total cholesterole, mM/l	5,19±0,65	4,585±0,59*	5,18±0,47	4,58±0,5 *
Triglycerides, mM/l	2,57±0,42	2,23±0,37*	2,51±0,41	2,06±0,34*
HDL, mM/l	1,18±0,14	1,32±0,10*	1,16±0,11	1,22±0,06*
LDL, mM/l	2,84±0,56	2,24±0,47*	2,87±0,41	2,42±0,47*
VLDL, mM/l	1,16±0,20	1,01±0,17*	1,15±0,19	0,94±0,15*
Atherogenic index of plasma (AIP)	3,47±0,82	2,48±0,44*	3,5±0,64	2,76±0,49*

Note: * - significant difference with appropriative indexes before treatment, $p \leq 0,05$

Table III. Influence of treatment on biochemical blood parameters which characterize the liver and kidneys state

Laboratory parameter	Sub-group 1A (n=28)		Sub-group 1B (n=27)	
	Before treatment	After 3 month treatment	Before treatment	After 3 month treatment
ALT, IU/l	27,68±4,96	21,72±3,57*	27,4±8,61	25,24±6,99
AST, IU/l	23,4±5,17	18,54±3,75*	22,06±5,26	21,57±5,17
GGT, IU/l	21,96±5,32	16,63±3,99*	21,3±12,68	19,0±13,34
Alkaline phosphatase (ALP), IU/l	44,14±13,56	33,71±12,14*	38,41±15,73	35,3±13,94
Total protein, g/l	71,19±3,8	75,38±2,34*	70,11±5,57	66,16±3,01*
Bilirubin total, mkM/l	13,5±2,83	12,25±2,04	13,03±4,08	12,96±3,32
Bilirubin direct, mkM/l	3,98±0,77	3,7±0,55	3,7±0,61	3,64±0,50
Creatinine, mkM/l	76,7±11,74	76,89±6,18	78,42±25,5	75,97±9,99
Uric acid, mM/l	285,9±56,9	245,7±41,91*	269,3±90,02	268,4±90,57

Note: * - significant difference between parameters before and after treatment, $p \leq 0.05$

perglycemia). Exclusion criteria were viral, autoimmune, alcohol, and toxic liver disease, cardiovascular diseases decompensation, infectious diseases (including tuberculosis), and psychiatric diseases.

All patients were laboratory examined. Complex biochemical blood test included such indexes as total bilirubin level and its fractions, uric acid, total protein, albumin, creatinine rates; enzymes activity (alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), gamma-glutamate transpeptidase (GGT), alkaline phosphatase (ALP). The lipid profile investigation included blood levels of total cholesterol (TC), triglycerides (TG); the high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) cholesterol; atherogenic index of plasma (AIP) calculation. The carbohydrate metabolism was evaluated by fasting and postprandial blood glucose levels (oral glucose tolerance test – OGTT), glycated hemoglobin (HbA1C). Insulin resistence (IR) was evaluated by IR-HOMA index according to the formula IR-HOMA = (fasting blood glucose X fasting blood insulin) / 22.5, where X means multiplication and / means division.

The average laboratory tests in investigated group were calculated before the treatment start (baseline) and compared to ones in control group and to average laboratory indexes in patients with NAFLD and concomitant DM-2.

Medical treatment of all patients included life style modification with moderate exertion (walking for at least 30 min every day) and diet correction (5 food intakes daily: 3 large meals and 2 small ones; limited fast-digesting carbohydrates and animal fats consumption, enough water use, 15% deficit in daily caloric intake). According to prescribed pharmacotherapy all patients with NAFLD and concomitant pre-diabetes (n=55) were divided into 2 sub-groups. Sub-group 1A included 28 patients that received EPL (medicine Essentiale Forte H) 2 capsules TID and omega-3 PUFA 1000 mg daily. Sub-group 1B included 27 patients that received rosuvastatin (10 mg daily) for dyslipidemia correction. The efficacy of different combinations was evaluated after 3 month treatment by comparison the average laboratory tests with respective baseline tests. The remote consequences were estimated 12 months after the start of combined treatment by laboratory tests comparison to baseline, 3-month results and control group results.

Table IV. Laboratory characteristics of long-term treatment results of patients with NAFLD and concomitant pre-diabetes

Groups, The number of patients in the group (n)	Sub-group 1A (n=28)	Sub-group 1B (n=27)	Group 3 (healthy) (n=30)
Laboratory test, units			
Fasting glucose, mM/l	5,52±0,48*	5,36±0,57*	4,08±0,59
HbA1C, %	5,78±0,20*	5,8±0,27*	5,13±0,44
IR-HOMA, U	3,44±0,87	4,47±1,18	2,33±0,23
Total cholesterol, mM/l	4,22±0,40*	4,15±0,35*	4,03±0,66
Triglycerides, mM/l	1,72±0,29*	1,55±0,14*	1,84±0,26
HDL, mM/l	1,36±0,07 *	1,25±0,05 *	2,27±0,76
LDL, mM/l	2,07±0,29 *	2,2±0,32 *	2,71±0,34
VLDL, mM/l	0,79±0,13 *	0,71±0,06 *	0,83±0,13
Atherogenic index of plasma (AIP), U	2,1±0,28 *	2,33±0,33 *	2,15±0,43
ALT, IU/l	16,7±2,65 *	24,07±6,42 #	12,56±3,12
AST, IU/l	14,0±2,26 *	20,46±5,20 #	8,32±2,89
GGT, IU/l	13,0±2,48 *	17,86±9,37	10,24±3,61
Alkaline phosphatase (ALP), IU/l	26,8±8,98 *	36,9±15,84	46,57±7,13
Total protein, g/l	76,2±1,82 *	63,56±3,03 *	73,86±2,56
Bilirubin total, mkM/l	10,65±1,31 *	12,27±2,21	10,01±1,24
Bilirubin direct, mkM/l	3,49±0,47 *	3,68±0,55	3,4±0,76
Creatinine, mkM/l	77,4±7,27	75,3±9,16	78,91±8,22
Uric acid, mM/l	234,1±41,73 *	256,8±77,03	280,21±22,3

Note: * - significant difference with parameter before treatment; # - significant difference with healthy, $p \leq 0.05$

The statistical analysis included Microsoft Excel table base of patients and Statistica 10.0 software use. Treatment group differences were evaluated using the independent sample t-test in case of normal distribution and Mann-Whitney test for non-parametric data. The significance of evaluated indices changes after treatment in normal distribution was evaluated using the Student's two-tailed test and in case of non-normal distribution – the Wilcoxon's test respectively. The difference was considered as significant in $p < 0,05$. All quantitative indices were presented in (M ± m) design where M is a mean group value and m is its mean deviation.

RESULTS

The results of this study demonstrate the laboratory changes in blood biochemical profile of patients with NAFLD and pre-diabetes (Table I).

This group of patients has not only disturbed carbohydrate metabolism, manifested by a significant increase in fasting glucose (6.26 ± 0.65 mmol / l) and the level of glycosylated hemoglobin (6.04 ± 0.24%) compared with the group of healthy individuals (respectively 4.08 ± 0.59 mmol/l and 5.13±0.44%), which makes it possible to diagnose pre-diabetes, but also insulin resistance of peripheral tissues as demonstrated significantly higher index IR-HOMA (5.50±2.92) in comparison with the healthy group (2.33±0.23). The activity of alanine aminotransferase (27.55±6.93 IU/l) and aspartate aminotransferase (22.74±5.17 IU/l), which were significantly higher than the corresponding indicators of healthy subjects in 2.2 and 2.73 times, indicated the presence of damage to hepatocyte membranes, which may lead to the development of a laboratory cytolysis syndrome. The presence of background for the occurrence of cholestasis is evidenced by the detected increase in GGTP activity, although the differences with healthy persons was not significant. There were no significant differences in the levels of total protein, bilirubin and its fractions, as well as the level of creatinine in patients with NAFLD compared to the control group of healthy individuals. Therefore, according to the results of this study, it cannot be stated that there is a disorder of the protein-synthetic function of the liver and pigmentary metabolism changes in patients with NAFLD and concomitant pre-diabetes. Renal dysfunction is also not confirmed.

The results of this study showed the clinical effectiveness of the use of essential phospholipids and omega-3 PUFA. The positive effect of both medicinal regimens (groups 1A and 1B) on carbohydrate metabolism was revealed: there was a decrease in blood glucose level, glycosylated hemoglobin and a decrease in insulin resistance index, however, these changes were not significant (Table II). In both experimental subgroups, there were positive changes in the lipid profile, all indicators significantly changed compared with the baseline, without a significant difference in lipid profiles indexes between these groups after treatment. This fact testifies that the medicine regimen with including EFL and omega-3 PUFAs resulted in the same changes in lipid metabolism as the use of 10 mg rosuvastatin daily (Table II).

According to the results presented in Table III, the use of EFL and omega-3 PUFAs (subgroup 1A) resulted in a decrease in the activity of ALT by 21.5%, AST by 20.77%, GGTP by 24.3%, alkaline phosphatase – by 23.6%, and these changes were reliable ($p<0.05$).

Positive changes in laboratory parameters were observed not only immediately after the end of the 3-month course of treatment, but also after 12 months, that indicated the stability of the therapeutic effect (Table IV). At the same time, no significant changes were found between the laboratory tests results of healthy persons and patients in group 1A in 12 month after treatment. In group 1B, the activities of ALT and AST in 12 months after treatment significantly differed from those of the healthy subjects. This fact indicates that there was no hepatoprotective effect in rosuvastatin.

DISCUSSION

Dyslipidemia was shown by significant changes in our patients triglycerides level, HDL cholesterol level, atherogenic factor and 22.2% higher compared to healthy person total cholesterol ones. The data is similar to results of other clinical studies [2, 8] and confirms the statement of current recommendations for diagnosis and treatment NAFLD (EASL-EASD-EASO, 2016) [6].

The prescription of EFL and omega-3 PUFA led to changes in liver blood tests of our patients. The revealed dynamics means positive hepatoprotective effect, which manifests itself in decreasing the intensity of the cytolysis and cholestasis syndromes. In addition, a significant increase ($p<0.05$) of the total protein level was observed under the influence of the combination of EFL and omega-3 PUFA, whereas under the influence of rosuvastatin it was significantly lowered. It probably means a positive influence of the combination of EPL and omega-3 PUFA on the liver protein-synthetic function. The same assumption was made by researchers [3, 9], who obtained similar application results. A significant decrease of uric acid was also found, that indicates positive effect of the combination of EPL and omega-3 PUFA on purine metabolism in patients with NAFLD and concomitant pre-diabetes. Similar changes of urine acid level under influence of same treatment was not declared in literature.

Consequently, according to results of our study, along with a hypolipidemic effect that does not significantly differ from the effect of rosuvastatin, the combination of an EFL with an omega-3 PUFA has a pronounced hepatoprotective effect. This fact gives grounds for recommending its use for patients with NAFLD in order to prevent the transition of hepatosteatosis (reverse stage) to steatohepatitis (irreversible stage).

Positive distant effects of treatment are explained, most likely, by the fact that during first 3 months of treatment patients develop a habit of leading the modified lifestyle and keeping on an adjusted diet, which remains after the end of the 3-month course of pharmacotherapy.

CONCLUSIONS

Along with the modification of lifestyle and dietary correction, it is advisable to prescribe medication for patients with non-alcoholic fatty liver disease and concomitant pre-diabetes. Inclusion of omega-3 polyunsaturated fatty acids and essential phospholipids into the therapeutic complex leads to an improvement in the laboratory pattern of these patients because it promotes not only correction of laboratory syndromes of cytolysis and cholestasis, as well as improvement of the glycemic profile, but also corrects dyslipidemia. Comprehensive treatment with the inclusion of these medicines can serve as a good alternative to rosuvastatin, since along with the hepatoprotective effect, it is characterized by both hypolipidemic and hypotriglyceridemic effects.

REFERENCES

1. Takahashi Y., Fukusato T. Histopathology of nonalcoholic fatty liver disease / nonalcoholic steatohepatitis. World Journal of Gastroenterology. 2014;20(42):15539–15548. doi: 10.3748/wjg.v20.i42.15539.
2. Schleicher J., Guthke R., Dahmen U. et al. A theoretical study of lipid accumulation in the liver-implications for nonalcoholic fatty liver disease. Biochimica et Biophysica Acta (BBA). 2014;1841(1):62–69. doi: 10.1016/j.bbalip.2013.08.016.
3. Stepanov Yu.M. The ese of essential phospholipids for the treatment of fatty liver disease. Gastroenterology. 2016;4(62):58-64. doi:10.22141/2308-2097.4.62.2016.81089.
4. Newsome P.N., Cramb R., Davison S.M. et al. Guidelines on the management of abnormal liver blood tests. Gut. 2018;67:6–19. doi:10.1136/gutjnl-2017-314924.
5. Patel P.J., Hayward K.L., Rudra R. et al. Multimorbidity and polypharmacy in diabetic patients with NAFLD: Implications for disease severity and management. Medicine (Baltimore). 2017;96(26):e6761. doi: 10.1097/MD.0000000000006761.
6. EASL–EASD–EASO. Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388–1402. doi: 10.1016/j.jhep.2015.11.004.
7. Argo C.K., Loria P., Caldwell S.H. et al. Statins in liver disease: A molehill, an iceberg, or neither? Hepatology. 2008;48(2):662–669. doi: 10.1002/hep.22402.
8. Berlanga A., Guiu-Jurado E., Porras J.A. et al. Molecular pathways in non-alcoholic fatty liver disease. Clinical and Experimental Gastroenterology. 2014;7:221–239. doi:10.2147/CEG.S62831.
9. Dajani A., Abu Hammour A. Treatment of Nonalcoholic Fatty Liver Disease: Where do we Stand? An Overview. Saudi Journal of Gastroenterology. 2016;22(2):91–105. doi: 10.4103/1319-3767.178527.
10. Gundermann K.J., Gundermann S., Drozdzik M. et al. Essential phospholipids in fatty liver: a scientific update. Clinical and Experimental Gastroenterology. 2016;9:105–117. doi:10.2147/CEG.S96362.
11. Basu P.P., Shah N.J., Aloysius M.M. et al. Effect of Vitamin E and Alpha Lipoic Acid in Nonalcoholic Fatty Liver Disease: A Randomized, Placebo-Controlled, Open-Label, Prospective Clinical Trial (VAIN Trial). Open Journal of Gastroenterology. 2014;4:199-207. doi:10.4236/ojgas.2014.45030.
12. Padma L., Mukaddam Q., Trailokya A. An Observational Study of Essentiale-L in the treatment of Patients with Fatty Liver Disease. Indian Journal of Clinical Practice. 2013;23:735–739.

13. Di Minno M.N., Russolillo A., Lupoli R. et al. Omega-3 fatty acids for the treatment of non-alcoholic fatty liver disease. World Journal of Gastroenterology. 2012;18:5839-5847. doi: 10.3748/wjg.v18.i41.5839.

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ORCID and contributionship:
Snizhana V. Feysa: 0000-0002-5064-8222 [A,B,C,D,F]
Svitlana O. Rudakova: 0000-0001-9129-4523 [E,F]

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CORRESPONDING AUTHOR
Snizhana V. Feysa
Uzhhorod National University
148 Sobranecka st., 88017 Uzhhorod, Ukraine
tel.: +380505600847
e-mail: snizhana.feysa@uzhnu.edu.ua

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ORIGINAL ARTICLE

EXPERIMENTAL STUDY OF BONE REBUILDING IN THE PERIIMPLANTATION AREA UNDER IMMEDIATE LOADING ON DENTAL IMPLANTS

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Anatoliy M. Potapchuk[1], Yevhen L. Onipko[1], Vasyl M. Almashi[1], Ninel V. Dedukh[2], Oleksandr Ye. Kostenko[1]
[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]STATE INSTITUTION "D. F. CHEBOTAREV INSTITUTE OF GERONTOLOGY, NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE", KYIV, UKRAINE

ABSTRACT

The aim: Study of the dynamics of morphological rearrangement of bone under conditions of immediate occlusive functional load and the effect of splinting of implants with temporary orthopedic structures with the analysis of the coefficient of stability of implants during immediate implantation in the experiment.

Materials and methods: A series of experiments was performed on 6 male Duroc pigs at the age of 6 months and weighing 40-60 kg. In the course of recent advances, the following methods have been used: the clinical protocol of immediate – implantation of time-consuming clothes, the definition of COEFICIENT, morphometry and light microscopy of the slides, statistical analysis.

Results: By morphometric examination after 3 months the BIC in the series with splinting was 1.68 times higher compared to 1 month. studies, in a series of experiments without splinting – 1.9 times, after 3 months. the difference between implantation experiments with splinting components and without splinting is 1.6 times. During the functional study of the resonant – frequency analyzer, there is an increase in the ISQ in the second and third months after surgery, but this figure is higher in the study using the splint component.

Conclusions: Stagnation of the shingle component in the case of intrinsic intraoperative functional juvenile implantation accelerates the dynamics of osteointegration, so that high indicators of the efficiency of the implant stability can be achieved.

KEY WORDS: Immediate – implantation, immediate loading, splinting, experiment, ISQ (Implant Stability Quotient), BIC (Bone to Implant Contact)

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INTRODUCTION

As a result of monitoring and analysis of clinical and experimental studies of dental implantation success in early and long periods of observation, scientists were able to justify the feasibility of introducing into clinical practice not only classical but also modified implantation protocols with different subsequent loading times [1, 2]. At the 6th ITI Consensus Conference (International Team of Implantologists), held in Amsterdam in April 2018, a group of scientists led by German Gallucci developed a unified classification of protocols for the installation and loading of dental implants for all possible clinical situations. Based on the analysis of publications selected for monitoring that met the criteria of evidence, the calculation of implant survival rates was made to determine the success and predictability of each protocol. Immediate implantation with immediate loading (type IA) can be performed only by specialists with high manual capabilities and with extensive clinical experience in the presence of certain clinical conditions with the provision of primary fixation of the torque 25-40 N / cm, or ISQ> 70 c.u. (conventional units) provides protection of occlusal functioning of temporary restorations [3]. Today there is a tendency to reduce the duration of subsequent orthopedic rehabilitation. Immediate implantation and immediate intraoperative functional masticatory load can reduce the duration of treatment and the volume of surgery and obtain a high functional and aesthetic result [4]. Today there are discussions about the peculiarities of the formation and level of reduction of peri-implant bone tissue in different conditions, in particular, under load and micromobility [5]. It was found that around the implants with load there is an increase in blood flow and vasomotor activity of the microcirculatory tract of supporting tissues, increase in bone volume and increase in torque, which is the optimal prognosis for accelerating the pace of osteointegration [6]. In the process of studying the dynamics of osseointegration, it was found that one of the reasons for implant disintegration is not timely loading, according to Professor Branemark, but the micro-mobility of the implant, which occurs as a result of this load [7]. Modern systematization of views on the differentiation of results of immediate loading and immediate implantation and their interpretation in terms of modern understanding of bone remodeling mechanisms expands the possibilities for discussion and argumentation of different protocols of dental implantation taking into account the initial conditions of the clinical situation [8]. Understanding the negative and early timing of implant loading under the influence of functional occlusal loading in conditions with expert analysis of morphological rearrangement of bone

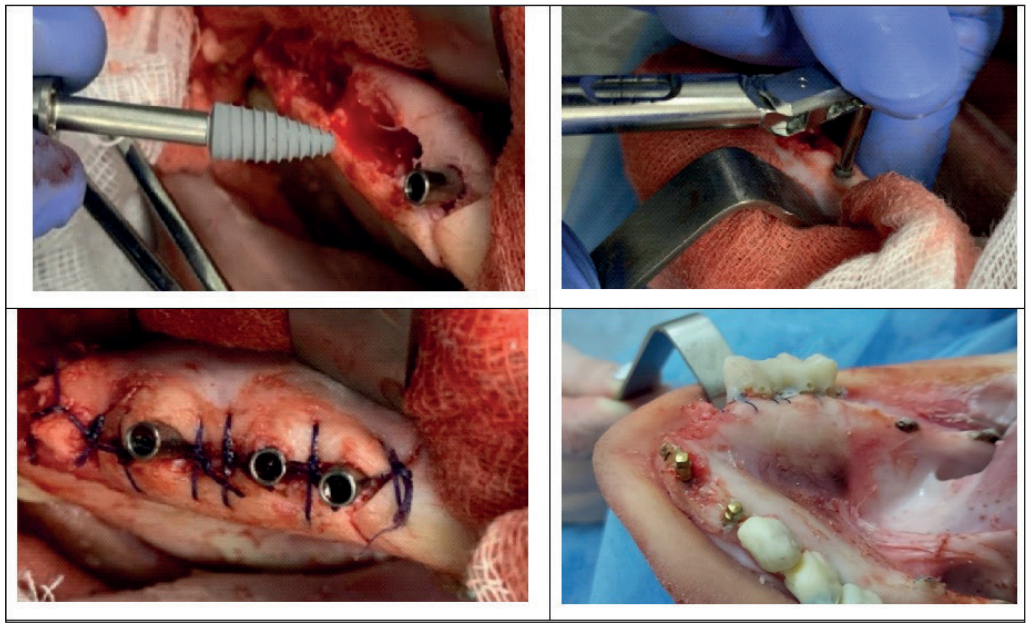

Fig .1. Stages of implantation (Art Implant, Ukraine)

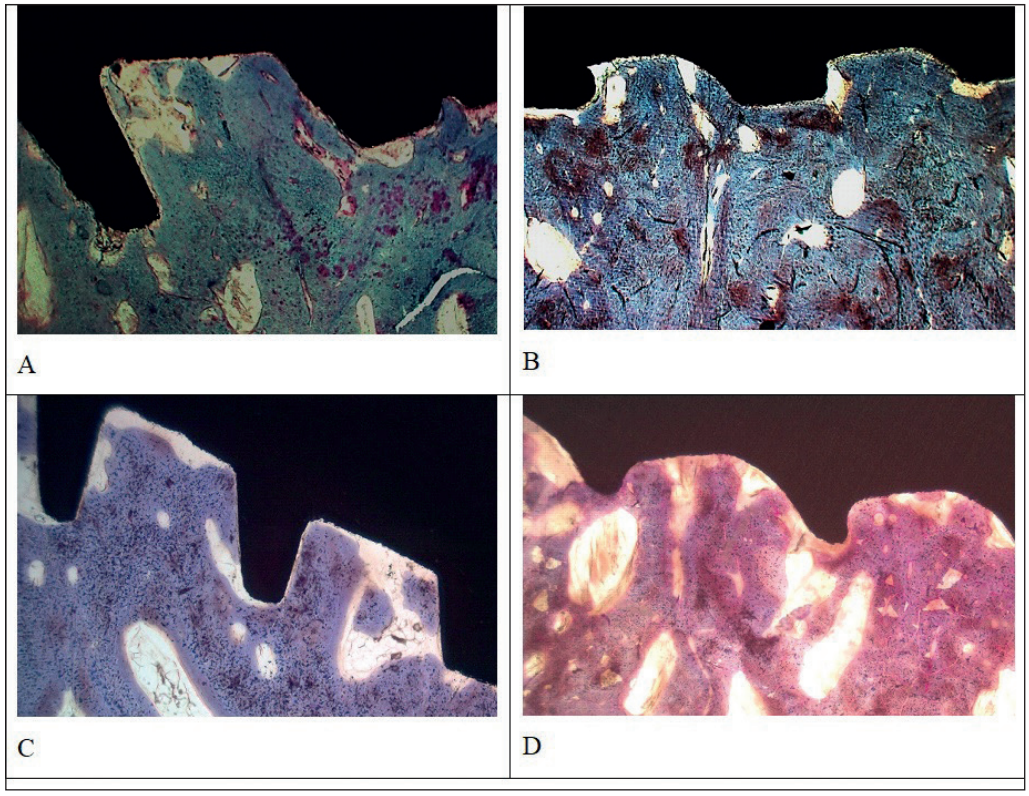

Fig. 2. Sections with implant area. Areas of bone and connective tissue in the apical (A) and cervical regions (B) of implants with and without splinting components in the apical (C) and cervical regions (D) of the implants Stained with hematoxylin and eosin. X 200.

tissue around implants is limited in basic research. There is also a lack of convincing experimental data to study the dynamics of osteogenesis at different times.

THE AIM

Study of the dynamics of morphological rearrangement of bone under conditions of immediate occlusive functional load and the effect of splinting of implants with temporary orthopedic structures with the analysis of the coefficient of stability of implants during immediate implantation in the experiment.

MATERIALS AND METHODS

Operation on animals. A series of experiments was performed on 6 male Duroc pigs at the age of 6 months and weighing 40-60 kg. The study was conducted in accordance with the general ethical requirements for the use of vertebrates in medical and biological experiments [10] and approved by the local committee on bioethics of Uzhhorod National University. Under the combined anesthesia (hexinal 5% -1g, relanium 10 ml, thiopental 0.5 g per 10 ml of saline) and infiltration anesthesia (Sol.Ultracain 2%) in the holes of the removed premolars and mandibular canines implants of the Art Implant system were installed (Ukraine,

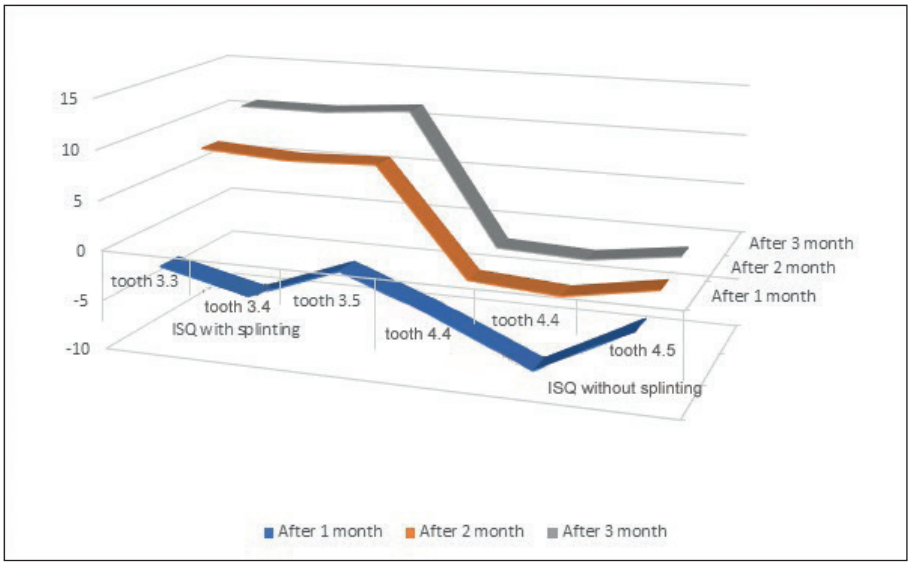

Fig. 3. Dynamics of stability of implants in the lower slot behind the Osstell Mentor for three months.

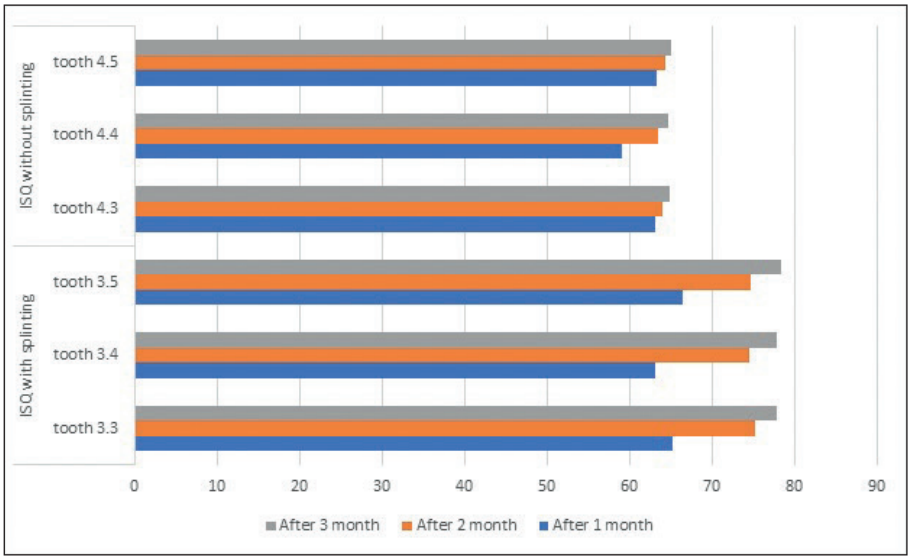

Fig. 4. Comparative dynamics of the dependence of the coefficient of stability of implants on the mandible according to Osstell Mentor on the use of the splinting component and without splinting in the clinical protocol of implantation.

certificate of conformity № UA.TR.101-87 / SU-1019). This is a two-stage dental implant, the endosal part of which is made in the shape of a cone. The external thread is made along its entire length, gradually increasing from 0.16 mm in the cervical to 9.9 in the apical part, with a thread pitch of 1.25 mm. The profile of the threaded turns is made trapezoidal-clamping with an angle of 15 degrees (Fig. 1).

Both implantation protocols provided occlusal functional loading. On the one hand (I series of researches – 3 implants) additional installation of a temporary splinting orthopedic design was carried out that provided functional stability of implants and elimination of micromovements of each implant, on the other hand jaws (II series of researches – 3 implants) temporary orthopedic designs were not separate and provided functional stability for the micromobility of implants.

The animals received a dairy-vegetable diet for 5 days after surgery. Observations of the condition of tissues in the area of the operation were performed daily for two weeks, then until the end of the experiment – once a week.

Determination of the stability coefficient of implants. The Implant Stability Quotient (ISQ) was determined using an Osstell Mentor resonant-frequency analyzer (Integration Diagnostics, Sweden). The primary stability of the implants was also measured intraoperatively with a torque wrench from the Vitaplant system (Germany) with a measuring range from 10 to 40 N / cm in steps of 5 N / cm. ISQ (Implant Stability Quotient) was performed using an Osstell Mentor resonant-frequency analyzer (Integration Diagnostics, Sweden). The primary stability of the implants was also measured intraoperatively with a torque wrench from the Vitaplant system (Germany) with a measuring range from 10 to 40 N / cm in steps of 5 N / cm.

Production of sections. Animals were removed from the experiment 1 and 3 months after surgery. From the lower jaw of the animals were cut bone fragments with implants for the manufacture of sections. The samples were placed in saline according to the Technovit system, in 4% neutral formaldehyde solution. The dehydrated bone block was placed in a special form with positioning on the orientation of the cut plane. Epoxy resin was used under vacuum for 15 minutes to fix (StruersClioVac). The next day, 1 mm thick fragments were isolated using a hard tissue microtome

Table I. Morphometric parameters of bone tissue on the surface of implants (M ± m, BIC,%) with and without splinting by loading after 1 and 3 months after implantation

Investigated indicators	The bone to implant contact (BIC)	
	1 month	3 months
Implants with splinting components	44.8± 3.19	75.27±2.98
Implants without splinting components	24.25± 1.36	46.87± 2.387

*Note: Significant difference between the indicators in BIC between groups implants with and without splinting components for 1 and 3 months, and in terms of terms ($p < 0.05$, for U-test Manna -Whitney).

(Leitz 1600) along the longitudinal axis and glued to the slide surface using thermoplastic adhesive (IKAC-MAGHP 4). Grinding the slices allowed to thin them to 20 microns (StruersLabopol 35). Subsequently, the resulting sections were stained with hematoxylin and eosin. Qualitative characteristics of bone tissue (morphometric studies) were described in a light microscope "Optika B-383PL 40x1000xTrino" (approx. 10, vol. 20). Histological specimens were photographed with a digital camera "SIGETA M3 CMOS 14 MP USB 3.0".

Morphometric analysis of the bone-implant interface. The bone to implant contact (BIC, %) was determined in the preparations to assess the osseointegration. Morphometric studies were performed on the photographs taken under one magnification using Micros microscope (Austria) (x 200) and DSM 800 camera software (Ukraine). Morphometric studies were performed according to the recommendations of Albrektsson T, & Wennerberg A. [9].

Statistics. Numerical values were presented as mean (M) and its standard error (m). After testing the series by the Kolmogorov-Smirny method, numerical values of the implant stability coefficient were processed using the Wilcoxon T-criterion for paired samples at 1, 2, and 3 months. The Mann-Whitney test was used to assess the reliability of implant stability coefficient values when comparing groups of animals with and without splinting in two independent samples, as well as to compare indicators in the morphometric study (series of experiments at 1 and 3 months). The results of laboratory and clinical studies were processed by the methods of variational statistics with determination of the average value, its errors, the Student's t test for multiple comparisons, using Excel (MS Office 2010, Microsoft, USA) and STATISTICA 6.0 (StatSoft, USA). Differences of indicators at significance level $p < 0.05$ were considered statistically significant.

RESULTS

Observations of the animals showed that after implantation, the healing of the wound was without complications, and no inflammatory reaction or suppuration was recorded.

Histological studies. One month later, around the implants with and without splinting components immersed in the lower jaw of pigs, the process of osteogenesis prevailed in the cervical region compared to the areas in the apical region. In the conditions of splinting in the implant neck area, the newly formed bone tissue penetrated between the threads in most areas, which contributed to the formation of a tight contact at the bone-implant interface. The foci of connective tissue were found, located along the convex part of the thread of the implant surface and in the recesses. When evaluating implants without splinting components in the endosal part of the implant revealed enlarged areas of connective tissue, alternating with small foci of newly formed bone tissue. The density of osteocytes in the bone tissue was greater around the implants with splinting. The cells were located in narrow lacunae. In the conditions of implantation without splinting, osteocyte lacunas were enlarged. Areas of bone tissue demineralization were revealed.

In the area of the thread of the implants with and without splinting, the adjacent bone tissue had characteristic signs of remodeling. Foci of overlaying of the newly formed bone tissue on the maternal bone, resorptive cavities of different sizes filled with loose connective tissue, which occupied large areas in the series of the experiment without the splinting component, were revealed. For this period of study, the BIC of implants with splinting was small, but compared with implants without splinting, the BIC was 1.85 times greater (Table I).

Three months after implantation, the area of bone tissue around the implants with splinting components were significantly larger compared to implants without splinting (Fig. 2).

After 3 months of the study in the series of implants with splinting the BIC was increased 1.68 times in comparison with 1 month, in the series of experiments without splinting – 1.9 times. The BIC difference of the implants with splinting components was 1.6 times more for this period compared to the implants without splinting (Table 1). Areas of connective tissue were present in both series (Figs. A, B, C, and D). As at the previous term, the localization of such areas was predominant in the experiment series without splinting.

Frequency-resonance analysis in determining the coefficient of stability of implants. Having stagnated the nonparametric statistical T-criterion of Vilcoxon, we improved the dynamics of the stability of the implants on the lower slit behind the Osstell Mentor because of a flexible component and without a single prototype in the basic protocol In the course of the follow-up carried out for the additional frequency – resonance analysis, we took away the onset results and the efficiency of the stability of the implants on the lower slit (Fig. 3).

The obtained results show that compared to the initial data of ISQ at the time of implantation, its values decrease sharply during the first month, so for tooth 3.4 (with splinting component) the difference is -3.96 ± 1.66 (p <0.02), and for tooth 4.4 (without splinting component), this difference is equal to the disintegration value of the implant – -8.14 ± 1.57 (p <0.02). From the second month of the study there is a moderate increase in the value of CSR, which is explained by the peculiarities of the dynamics of morphological changes in osteointegration processes and is consistent with the data of other authors [11]. According to the analysis of numerical indicators according to the Mann-Whitney U-test, which is used to assess the difference between two unrelated samples (in the following case, between implants with splint components and without splints for periods of 1.2 and 3 months. results (Fig. 4).

The results show that during the first month after implantation with the splint component there was a decrease in stability in the bone-implant connection, the average value of ISQ in the study for tooth 3.3 was 65.23 ± 2.08, for tooth 3.4 – 63.12 ± 1.88, and for tooth 3.5 – 66.44 ± 2.12 (p <0.03); in the study without a tire, these values were respectively: for tooth 4.3 – 63.04 ± 2.08, for tooth 4.4 – 59.08 ± 2.12, and for tooth 4.5 – 63.23 ± 2.08 (p <0, 03), which corresponds to the micromobility of the implant. There is an increase in the stability of the implant in the second and third months after surgery, but this figure is higher in the study using the splint component. The average value of ISQ for tooth 3.3 in the second month of implantation is 75.16 ± 3.31 (p <0.04), tooth 3.5 in the third month of implantation – 78.42 ± 4.75 (p <0.05).

DISCUSSION

One of the most important conditions in dental implantology is the creation of conditions for successful long-term and stable fixation of implants. The success of the implantation of non-biological implants into the bone depends on osseointegration, that is, the formation of a direct structural-functional connection between the implant and the bone. Data on the peculiarities of osseointegration, processes of the state of bone tissue, formation and resorption of bone around dental implants made of metals with different surfaces and qualities attract the attention of specialists in connection with the development of various protocols for installation and loading [12]. The process of osseointegration is a regulated cascade of intracellular and extracellular biological mechanisms, in the implementation of which various modes and periods of exercise play a significant role [13]. Fundamental studies of the state of the bone tissue around the implanted material are important for assessing the optimal timing of the early functional load on the implant, since the early loading function contributes to an increase in osseointegration; however, in the complex context of bone remodeling, disintegration of the implant is also possible. There is a study in which 29 articles were selected out of 889 meeting the inclusion criteria, based on the assessment of various loading protocols (immediate, immediate non-occlusive, early and normal loading) of dental implants for marginal bone loss [14]. The lowest level of marginal bone loss around implants was recorded with immediate loading (0.05 ± 0.67 mm), and the highest for implants with immediate non-occlusive loading (1.37 ± 0.5 mm), that is. immediate loading protocol is a reasonable alternative to traditional loading protocol. In our study, under the conditions of implantation in the lower jaw of the bone of pigs Art Implant (Ukraine), studies were carried out in two directions – with splinting components and without splinting, with an assessment of the state of the peri-implantation bone by a histological method and using the technology for determining the stability coefficient. There are two types of osteogenesis – contact, on the surface of the implant, and remote, which takes place in the peri-implantation area, and behind the researchers' data, the remotely located bone delivers signals that induce contact osteogenesis [15]. In this regard, two indicators are usually used to assess osseointegration in histological studies – BIC and an assessment of bone tissue formation at a distance from the implant [16]. The latter indicator can also reflect the effect of the implantation on the surrounding bone. In our study, we have demonstrated that under conditions of direct loading and stabilization, there is a significant increase in bone tissue on the screw surface of the implant and in the area adjacent to the thread. Decreases osteocytic osteolysis, which prevents bone demineralization. In the absence of splinting with physically conditioned micro-mobility of implants during loading, resorptive processes in the bone tissue increase both on the surface of the implant and in the areas adjacent to the screw region, extensive areas of demineralization are found, which affects the contact of the implant with the bone. We can assume that our two-stage intraosseous dental implant provides low-trauma insertion of the implant with a self-tapping screw into the bone bed of all bone types, provides contact osteogenesis, seals the structures of the cancellous bone without disturbing the structure of trabeculae and precervical resorption in the wound. The morphological studies carried out were confirmed by the data using the dynamics of the stability coefficient of implants in the lower jaw according to Osstell Mentor in conditions of using a splinting component and without splinting in the clinical implantation protocol.

CONCLUSIONS

Stabilization of implants using a splinting component increases the direct contact of the implant with the surrounding bone due to the activation of osteogenesis in comparison with implants without splinting: after 1 month. BIC increases 1.9 times, after 3 months. – 1.6 times.

Stagnation of the shingle component in the case of intrinsic intraoperative functional juvenile implantation accelerates the dynamics of osteointegration, so that high indicators of the efficiency of the implant stability can be achieved.

REFERENCES

1. Tonetti M.S., Cortellini P., Graziani F. et al. Immediate versus delayed implant placement after anterior single tooth extraction: the timing randomized controlled clinical trial. J Clin Periodontol. 2017; 44(2):215-224.
2. Potapchuk A.M., Rusyn V.V., Onipko Ye.L. et al. Porivnyal'nyy analiz rivniv reduktsiyi peryimplantatnoyi kistkovoyi tkanyny pry realizatsiyi nehaynoho ta vidterminovanoho protokoliv dental'noyi implantatsiyi. Novyny stomatolohiyi. 2020: 6-12.
3. Gallucci G.O., Hamilton A., Zhou W. et al. Implant placement and loading protocols in partially edentulous patients: A systematic review. Clinical Oral Implants Research. 2018; 29(6):106-134.
4. Tarazona B., Tarazona-Álvarez P., Peñarrocha-Oltra D., Peñarrocha-Diago M. Relationship between indication for tooth extraction and outcome of immediate implants: A retrospective study with 5 years of follow-up. J Clin Exp Dent. 2014; 6(4):384-388.
5. Montero J., Fernández-Ruiz A., Pardal-Peláez B. et al. Effect of Rough Surface Platforms on the Mucosal Attachment and the Marginal Bone Loss of Implants: A Dog Study. Materials (Basel). 2020; 13(3):802.
6. Sheng L., Silvestrin T., Zhan J. et al. Replacement of severely traumatized teeth with immediate implants and immediate loading: literature review and case reports. Dental Traumatology. 2015; 31(6): 493-503.
7. Savranskiy F.Z., Simakhov R.V., Grishin P.O. et al. Osobennosti provedeniya neposredstvennoy implantatsii i nemedlennoy nagruzki pri primenenii implantatsionnoy sistemy "Humana Dental". Suchasna stomatologíya. 2018: 58-63.
8. Potapchuk A.,Rusyn V., Goncharuk-Khomyn M., Hegedus V. Prognosis of possible implant loss after immediate placement by the laboratorial blood analysis and evaluation of intraoperatively derived bone samples. Journal of International Dental and Medical Research. 2019; 12(1):143-150.
9. Albrektsson, T., Wennerberg, A. On osseointegration in relation to implant surfaces. Clinical Implant Dentistry and Related Research. 2019; 21: 4–7.
10. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasburg, 1986, ETS no. 123). https://rm.coe. int/168007a67b.
11. Sargolzaie N., Samizade S., Arab H. The evaluation of implant stability measured by resonance frequency analysis in different bone types. J Korean Assoc Oral Maxillofac Surg. 2019; 45(1): 29–33.
12. Zaid M.B., O'Donnell R.J., Potter B.K., Forsberg J.A. Orthopaedic Osseointegration: State of the Art. J Am Acad Orthop Surg. 2019; 27(22):977-985.
13. Duyck J., Vandamme K. The effect of loading on periimplant bone: a critical review of the literature. J Oral Rehabil. 2014; 41:783–794.
14. Sommer M., Zimmermann J., .Grize L., Stübinger S. Marginal bone loss one year after implantation: a systematic review of different loading protocols. International Journal of Oral and Maxillofacial Surgery. 2020; 49(1):121-134.
15. Choi J.Y., Sim J. H., Yeo I. L. Characteristics of contact and distance osteogenesis around modified implant surfaces in rabbit tibiae. Journal of Periodontal Implant Science. 2017; 47(3): 182–192.
16. Liu Y., Rath B., Tingart M., Eschweiler J. Role of implants surface modification in osseointegration: A systematic review. J Biomed Mater Res. 2020; 108A:470–484.

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ORCID and contributionship:
Anatoliy M. Potapchuk: 0000-0001-9857-1407 [A,E,F]
Yevhen L. Onipko: 0000-0002-3086-4657 [A,B,D]
Vasyl M. Almashi: 0000-0002-2943-4844 [B,C,E]
Ninel V. Dedukh: 0000-0003-0307-2328 [A,D]
Oleksandr Ye. Kostenko: 0000-0002-0549-1561 [D]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Anatoliy M. Potapchuk
Uzhhorod national university
60a Station St., 88000 Uzhhorod, Ukraine
tel: +380509399457
e-mail: anatoliy.potapchuk@uzhnu.edu

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ORIGINAL ARTICLE

ASSESSMENT OF CARDIOVASCULAR RISK IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ASSOCIATED OBESITY AND WAYS OF ITS CORRECTION

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Taras I. Griadil[1], Ivan V. Chopey[1], Ksenia I. Chubirko[1], Mykhaylo M. Hechko[1], Wael Rumaneh[2]
[1]STATE HIGHER EDUCATIONAL ESTABLISHMENT «UZHHOROD NATIONAL UNIVERSITY», UZHHOROD, UKRAINE
[2]SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

The aim: To analyze and calculate CVR in patients with T2DM and concomitant obesity.

Materials and methods: The selection of patients was carried out based on the Uzhhorod District Clinical Hospital, in the period from November 2016 to January 2020. All patients were divided into 3 groups: 1 (n=93) with T2DM and concomitant obesity, 2 (n=87) with T2DM, 3 (n=39) with obesity. The treatment period lasted 1 year and included dosed exercise for at least 30 minutes per day and dietary recommendations. Patients in groups 1 and 2 received metformin 850 mg twice daily in combination with dapagliflozin 10 mg once daily. CVR was determined at the time of enrollment and after 1 year of treatment using: American College of Cardiology / American Heart Association Guideline on the Assessment of Cardiovascular Risk (2013) (ASCVD Risk) and Framingham Risk Score (FRS).

Results: The data obtained as a result of the study revealed the highest CVR in patients of group 1, in contrast to group 2 and 3 ($p<0.05$). After 1 year of complex treatment, CVR indicators were statistically significantly reduced in all experimental groups ($p<0.05$).

Conclusions: Determining CVR parameters and exposure to them within 10 years can remove unwanted cardiovascular complications.

KEY WORDS: type 2 diabetes mellitus, obesity, treatment, diagnostics, dapagliflozin, cardiovascular risk

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by multivariate systemic complications that significantly impair the quality of life of patients and reduce their life expectancy [1].

T2DM is considered an absolute risk factor for atherosclerosis [2]. The basis of this pathological process is a violation of carbohydrate metabolism, one of its manifestations is hyperglycemia, which leads to changes in the lipid spectrum of blood (deviate from normal levels of total cholesterol, triglycerides, high and low-density lipoprotein cholesterol) [3]. In the long run, lipid-protein glycan complexes are deposited in blood vessels, leading to the development of diabetic micro- or macroangiopathy [3, 4].

Therefore, chronic microvascular complications of T2DM include nephropathy, erectile dysfunction, cataracts, and retinopathy, the consequences of which are blindness, neuropathy with a distant complication – amputation of limbs and others [5]. Instead, chronic macrovascular complications of T2DM include atherosclerotic lesions of the lower extremities resulting in the diabetic foot, which in turn may be complicated by the need for amputation of limbs, stroke, coronary heart disease (CHD) with a possible course: angina and/or myocardial infarction (MI) and others [6].

According to studies, patients with T2DM are overweight and obese [7]. Obesity from a pathophysiological point of view is considered as a chronic inflammatory process that has a complex and detrimental effect on the whole body in general, provoking the development of hypertension, impaired glucose metabolism, vascular damage (acceleration of atherosclerosis and its consequences), contributing to the development of cardiovascular complications (MI, stroke, etc.), infertility, oncology, obstructive sleep apnea syndrome, etc. [7, 8]. Several clinical studies have shown that anthropometric parameters are closely related to the risk of cardiovascular disease (CVD): body mass index (BMI), waist circumference (WC), hip circumference (HC), and waist-to-hip ratio (WHR) [9]. Each additional kilogram of weight directly affects the final value of blood pressure, while the increase in waist WC, HC, WHR is closely related to the predictor of CVD [10]. That is why obesity is considered an additional factor of cardiovascular risk (CVR).

Quite often there is a comorbid combination of T2DM and hypertension, which significantly accelerates the pathological process of vascular endothelial damage, both metabolic pathogenesis and changes in vascular pressure, which exacerbates the development of vascular complications of the kidneys and heart, brain, peripheral vessels of the lower extremities [11]. Thus, the risk of CHD, stroke and dozens of times, vision loss, amputation of the lower extremities, and other complications increases several times [11].

Cardiovascular complications as a consequence of T2DM can be prevented and in some cases removed by determining the patient's CVR, know the prognosis for the patient and make a comprehensive correction based on individual needs.

From a practical point of view, various methods of assessing CVR are widely used, in particular in the 10-year perspective: Q risk 2 score calculator and Modified Q risk 2, PROCAM score, The Framingham risk score (FRS), SCORE, American College of Cardiology / American Heart Association Guideline on the Assessment of Cardiovascular Risk (2013) (ASCVD Risk) [12-16].

SCORE, FRS, ASCVD Risk remain the most widely used in routine clinical practice to assess CVR. Thus, the SCORE scale, created based on the results of clinical trials involving more than 250,000 patients, allows us to assess the risk of fatal cardiovascular events (CVE) in the next 10 years. [13, 15, 16].

American College of Cardiology / American Heart Association Guideline on the Assessment of Cardiovascular Risk (2013) (ASCVD Risk) is categorized as low-risk (<5%), borderline risk (5% to 7.4%), intermediate-risk (7.5% to 19.9%), high risk (≥20%) of 10-year risk of MI and/or stroke [15, 17].

The Framingham Risk Score (FRS) for hard CHD which evaluates the ten-year risk of CVD (CHD, stroke, chronic heart failure, heart death) in percentage was calculated by total points was classified as low risk (<10%), intermediate-risk (10–20%), and high risk (>20%) [14, 18].

CVD is one of the leading causes of global mortality and one of the most common causes of disability. CVD prevalence increased from 271 million in 1990 to 523 million in 2019 and continues to rise, while the number of CVD deaths increased from 12.1 million in 1990 to 18.6 million in 2019 [19].

Annually increasing costs associated with CVD, particularly so in the USA alone as of 2015 spent 126 billion dollars and are projected to grow more than 2.5 times to 309 billion dollars in 2035 [20].

THE AIM

Analyze and calculate CVR in patients with T2DM and concomitant obesity and comprehensively influence the obtained CVR, reducing the 10-year risk of CVE.

MATERIALS AND METHODS

The selection of patients took place based on the therapeutic department of the Municipal Non-Profit Enterprise "Uzhhorod District Clinical Hospital of Uzhhorod District Council of Transcarpathian region", and at outpatient treatment department of the therapy and the family medicine of the Faculty of Postgraduate and Pre-University Education of the State Higher Educational Establishment «Uzhhorod National University» in the period from November 2016 to January 2020. In the course of the study, 93 people with T2DM and concomitant obesity, who were included in the 1st group, were examined and 126 medical cards of an inpatient with a diagnosis of T2DM and ambulatory card data included in the 2nd group were retrospectively analyzed. group (n=87), while group 3 included patients diagnosed with obesity (n=39). The treatment period in patients lasted 1 year and included dosed exercise lasting at least 30 minutes a day and dietary recommendations, also patients in groups 1 and 2 received metformin 850 mg 2 times a day in combination with dapagliflozin 10 mg 1 time per day.

All subjects were examined: general clinical examination, anthropometric measurements, calculation of BMI, WC, HC, WHR, glycosylated hemoglobin (HbA1c), lipid profile, collection of medical and social history, and bad habits. All patients in the study were additionally interviewed about the correctness of dietary and treatment recommendations.

CVR was determined at the time of inclusion in the study and after 1 year of treatment. The following calculators were used to calculate the CVR: 1) American College of Cardiology/American Heart Association Guideline on the Assessment of Cardiovascular Risk (2013) (ASCVD Risk) is categorized as low-risk (<5%), borderline risk (5% to 7.4%), Intermediate risk (7.5% to 19.9%), high risk (≥20%) of 10-year risk of MI and/or stroke [15, 17] and 2) The Framingham Risk Score (FRS) for hard CHD which evaluates the ten-year risk of CVD (CHD, stroke, chronic heart failure, heart death) in percentage was calculated by total points was classified as low risk (<10%), intermediate-risk (10–20%), and high risk (>20%) [14, 18].

Additionally, to find the potential risk for patients with T2DM, a bibliographic search was performed on the keywords "treatment of type 2 diabetes mellitus", "type 2 diabetes mellitus", "dapagliflozin", "metformin", "risk factors", "cardiovascular risk" in the following databases PubMed, MEDLINE, Web of Science, Cochrane Library, Google Akademy.

The diagnosis criteria for T2DM were established based on the American Diabetes Association. The diagnosis of obesity was established by measuring BMI≥30 kg/m^2, and the value of BMI was assessed by the degree of obesity.

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

The whole set of the surveys were by the Articles 3,44 of the Fundamentals of the Legislation of Ukraine on Healthcare, the Articles 7, 8 of the Law of Ukraine "On Medicines", the Law of Ukraine "On Protection of Personal Data", taking into account the requirements of the European Parliament and

Table I. Anthropometrical parameters in group 1, 2 and 3.

Parameter	Group		
	Group 1 (n=93)	Group 2 (n=87)	Group 3 (n=39)
BMI ^{B} (kg/m^2)	32,57±0,18	28,74±0,21	32,24±0,32
WC ^{B} (cm)	110,59±1,35	88,41±1,17	108,62±1,19
HC ^{B} (cm)	103,8±0,92	92,6±1,04	105,17±0,89
WHR ^{B}	1,07±0,01	0,95±0,01	1,03±0,01
BMI ^{AT} (kg/m^2)	31,34±0,22	27,32±0,17*	30,67±0,26#
WC ^{AT} (cm)	104,67±1,18	85,72±1,12*	103,70±1,08#
HC ^{AT} (cm)	101,2±1,04	90,3±1,19*	102,21±0,89#
WHR ^{AT}	1,03±0,01	0,95±0,01*	1,01±0,01#

Note: B - patient data at the beginning of the study; AT - patient data after 12 months of treatment and follow-up; BMI - Body Mass Index; WC - Waist circumference; HC - the hip circumference; WHR - waist-to-hip ratio; * - statistically significant difference when comparing the indicators between the respective groups 1 and 2 ($p<0.05$); # - a statistically significant difference when comparing the indicators between the respective groups 1 and 3 ($p<0.05$).

Table II. FPG and HbA1C levels

Parameter	Group		
	Group 1 (n=93)	Group 2 (n=87)	Group 3 (n=39)
FPG ^{B}	9,21±0,17	8,96±0,11	5,81±0,12
HbA1C (%) ^{B}	8,3±0,05	8,12±0,08	5,77±0,06
FPG ^{AT}	7,21±0,08	6,69±0,12*	5,51±0,15#
HbA1C (%) ^{AT}	7,51±0,03	6,45±0,04*	5,49±0,03 #

Note: B - patient data at the beginning of the study; AT - patient data after 12 months of treatment and follow-up; FPG – Fasting plasma glucose; HbA1C – glycated hemoglobin; normal values of FPG – 3.3-5.5 mmol/l; normal values of HbA1C – 4-6.4%; * - statistically significant difference when comparing the indicators between the respective groups 1 and 2 ($p<0.05$); # - a statistically significant difference when comparing the indicators between the respective groups 1 and 3 ($p<0.05$).

Council Directives 2001/20/ EU of April 4, 2001, 2001/83/ EU of November 6, 2001, the Decisions of the European Parliament and of the Council 1901/2006 of December 12, 2006, and 1902/2006 of December 20, 2006, ICH GCP, International Ethical Principles for Biomedical human-related research and physician code of conduct, and order in the Ministry of Health of Ukraine No. 690 of September 23, 2009, as well as the order of the Ministry of Health of Ukraine No. 1118 of December 21, 2012, the unified clinical protocol of the primary and the secondary (specialized) medical care of T2DM.

RESULTS

Those included in this study were ≥40 years old. The mean age of the patients in the 1st group was 51.3±1.2 years, compared with 52.7±1.1 years of the patients in the 2nd group, whereas in group 3 the age of patients was 54.5±1.2 years. The ratio of men and women in group 1 was 32 men and 61 women against 34 men and 53 women in group 2 and 18 men and 21 women in group 3. The mean duration of T2DM in group 1 was 14.8±3.2 years, as opposed to 13.5±1.6 years in group 2.

Currently, the status of a smoker was in group 1 – 29 people, group 2 – 17 people, and group 3 – 22 people. Instead, in the past, there were additionally smokers in group 1 – 13 people, in group 2 – 8 people, and group 3 – 6 people. Hypertensive disease and received treatment for it: in group 1 – 35 people, in group 2 – 21 people, and group 3 – 27 people. MI was suffered in the past: in group 1 – 11 people, in group 2 – 7 people, and group 3 – 4 people. Instead, during 1 year of observation, MI was additionally transferred: in group 1 – 3 persons, in group 2 – 1 person, and group 3 – 2 persons. Stroke was suffered in the past: in group 1 – 8 people, in group 2 – 3 people, and group 3 – 1 person. Instead, during 1 year of follow-up, an additional stroke: in group 1 – 2 people, in group 2 – 3 people, and in group 3 – 2 people. Aspirin therapy was taken: in group 1 – 36 people, in group 2 – 15 people, and group 3 – 11 people. Statins were taken: in group 1 – 24 people, in group 2 – 11 people, and in group 3 – 7 people.

At the beginning of the study, according to the obtained data on BMI: in group 1 – 58 people were with grade I obesity, 23 people had grade II obesity, 11 people had grade III obesity; in group II – 53 people were overweight, while 34 people were normal weight; in group 3 – 25 people were with I degree of obesity, 11 people had II degree of obesity and 3 people with obesity of III degree.

According to the data obtained as a result of the measuring anthropometric parameters of the patients of the 1st and the 2nd group and 1st and the 3rd group at the beginning of the study, no statistically significant difference was found between them ($p>0.05$). The BMI at the beginning of the study in group 1 was 32,57±0,18 kg/m2, respectively 28,74±0,21 kg/m2 in group 2 and 32,24±0,32 kg/m2 in group 3. The WC index in group 1 at the beginning of the study was 110,59±1,35 cm, respectively 88,41±1,17 cm in group 2, and 108,62±1,19 cm in group 3. WHR in the group 1 was 1,07±0,01 and 0,95±0,01, respectively in the group 2 and 1,03±0,01 cm in the group 3.

It is noteworthy that 12 months after the course of comprehensive treatment and observation, between anthropometric indicators of patients of the 1st and 2nd group there was a statistically significant difference, the same dynamics were also observed when comparing the 1st and 3rd groups ($p<0.05$).

If at the beginning of the study in group 1 HbA1C was 8.3±0.05%, then after 12 months of complex treatment and observation 7.51±0.03%, against the response of 8.12±0.08% and 6.45±0.04%, respectively, in the second group. In contrast, in patients of group 3 before and after 12 months of complex treatment and observation, indicators within the norm of HbA1C were observed – 5.77±0.06% and 5.49±0.03%, respectively. According to the obtained laboratory data of FPG and HbA1C, in patients of the 1st and 2nd groups and the 1st and 3rd groups at the begin-

Table III. Assessment of CVR on the American College of Cardiology/ American Heart Association Guideline on the Assessment of Cardiovascular Risk (2013) (ASCVD Risk)

Parameter	Group		
	Group 1 (n=93)	Group 2 (n=87)	Group 3 (n=39)
ASCVD Risk (%) [B]	12,7±0,1	11,8±0,2	6,8±0,2
ASCVD Risk (%) [AT]	9,5±0,1	9,2±0,3*	6,4±0,2#

Note: B - patient data at the beginning of the study; AT - patient data after 12 months of treatment and follow-up; CVR – cardiovascular risk; ASCVD Risk - assessment of cardiovascular risk; ASCVD Risk is categorized as low-risk (<5%), borderline risk (5% to 7.4%), intermediate-risk (7.5% to 19.9%), high risk (≥20%) of 10-year risk of myocardial infarction and/or stroke; * - statistically significant difference when comparing the indicators between the respective groups 1 and 2 ($p<0.05$); # - a statistically significant difference when comparing the indicators between the respective groups 1 and 3 ($p<0.05$).

Table IV. Assessment of CVR on the Framingham Risk Score (FRS)

Parameter	Group		
	Group 1 (n=93)	Group 2 (n=87)	Group 3 (n=39)
FRS (%) [B]	24,6±0,4	18,5±0,3	14,8±0,5
FRS (%) [AT]	21,5±0,4	16,1±0,4*	12,3±0,2#

Note: B - patient data at the beginning of the study; AT - patient data after 12 months of treatment and follow-up; FRS - Framingham Risk Score; FRS was classified as low risk (<10%), intermediate-risk (10–20%), and high risk (>20%) of ten-year risk cardiovascular disease; * - statistically significant difference when comparing the indicators between the respective groups 1 and 2 ($p<0.05$); # - a statistically significant difference when comparing the indicators between the respective groups 1 and 3 ($p<0.05$).

ning of the study, no statistically significant difference was found between them ($p>0.05$). Analyzing the biochemical parameters of the blood, namely the metabolism of hydrocarbons, there is a tendency to reduce the level of fasting plasma glucose (FPG) and HbA1C in groups 1 and 2. There was a statistically significant difference between FPG and HbA1C in patients of groups 1 and 2 and groups 1 and 3 after 12 months of study ($p<0.05$).

In all study groups, at the beginning of the study, there was an increased level of triglycerides, a decrease in high-density lipoprotein, and an increase in low-density lipoprotein. The level of triglycerides slightly decreased after treatment, compared with a baseline before treatment, but was still extremely high, a statistically significant difference between patients 1 and 2 groups and between patients 1 and 3 groups was not observed ($p>0.05$). In groups 1 and 2 at the beginning of the study, there was an increase in the concentration of apolipoprotein B over 120 mg/dl, while in groups 3 this figure was within normal limits. Targets of the lipid profile in the experimental groups after the course of treatment were not achieved.

The other biochemical parameters obtained at different stages of the study did not reveal the statistically significant changes in the indicators of the groups 1 and 2 and 1 and 3 ($p>0.05$).

At the beginning of treatment, ASCVD Risk in patients of group 1 was 12.7±0.1%, group 2 – 11.8±0.2%, and group 3 6.8±0.2%, respectively. At the end of treatment, ASCVD Risk in patients of group 1 was 9.5±0.1%, group 2 – 9.2±0.3%, and group 3, respectively, 6.4±0.2%. There was a statistically significant difference between ASCVD Risk, between patients in groups 1 and 2 and groups 1 and 3 after 12 months of study ($p<0.05$).

At the beginning of treatment, FRS in patients of group 1 was 24.6±0.4%, group 2 – 18.5±0.3%, and group 3 14.8±0.5%, respectively. At the end of FRS treatment in patients of the 1st group 21.5±0.4%, the 2nd group – 16.1±0.4%, and the 3rd group 12.3±0.2%, respectively.

Thus, after a comprehensive examination with the identification of risk factors for cardiovascular events and subsequent calculation of CVR, after a course of treatment, there was a tendency to decrease this indicator. However, in 12 months of treatment and follow-up, new episodes of CVE were recorded, which unfortunately could not be prevented. Therefore, patients of all study groups were provided with further treatment recommendations and advice on continuing lifestyle modifications followed by follow-up.

DISCUSSION

Even though many medical instruments help to individually assess the CVR in a 10-year period, covering several clinical and laboratory data of the patient, they remain quite rough instruments [16-18]. However, CVR scales do not include other equally important RF that may directly affect the CVE prognosis over a 10-year period [19]. Treatment recommendations are based on data from the CVR, are group character that can reduce the effectiveness of individual therapy.

Therefore, more individualized scales for assessing CVR are currently being developed. Future CVR scales on the way to personalized medicine may take into account individual genetic characteristics, which will significantly increase their sensitivity. New and individual CVR assessment scales may lead to a rethinking of treatment guidelines and significantly improve treatment outcomes.

CONCLUSIONS

Patients with T2DM and concomitant obesity have higher CVR rates compared with groups of patients with T2DM and obesity alone. It is also important that in the long run, in 10 years or more, the indicators of CVR can be corrected, which reduces the risk of the patient having unwanted CVE and potential disability of the patient.

The frequency of CVE can be reduced by providing patients with adequate comprehensive treatment and control of blood pressure and hydrocarbon metabolism, lifestyle modifications, and the like. However, new cardiovascular events may occur due to unmodified risk factors and/or insufficient exposure to modified risk factors.

REFERENCES

1. Naranjo C., Ortega-Jiménez P., Del Reguero L. et al. Relationship between diabetic neuropathic pain and comorbidity. Their impact on pain intensity, diabetes complications and quality of life in patients with type-2 diabetes mellitus. Diabetes research and clinical practice. 2020;165: 108236.
2. Poznyak A., Grechko A.V., Poggio A. et al. The diabetes mellitus–atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. International journal of molecular sciences 2020; 21(5): 1835.
3. Demirdogen R.E. Relationship among blood boron level, diabetes mellitus, lipid metabolism, bone metabolism and obesity: Can boron be an efficient indicator for metabolic diseases. Health Sci. J. 2020;14, 1-11.
4. Cole J.B., Florez J.C. Genetics of diabetes mellitus and diabetes complications. Nature Reviews Nephrology. 2020; 16(7): 377-390.
5. Faselis C., Katsimardou A., Imprialos K. et al. Microvascular complications of type 2 diabetes mellitus. Current vascular pharmacology. 2020; 18(2): 117-124.
6. Viigimaa M., Sachinidis A., Toumpourleka M. et al. Macrovascular complications of type 2 diabetes mellitus. Current vascular pharmacology. 2020; 18(2): 110-116.
7. Silveira E.A., de Souza Rosa L.P., de Souza Cardoso C.K. Type 2 diabetes mellitus in class II and III obesity: Prevalence, associated factors, and correlation between glycemic parameters and body mass index. International Journal of Environmental Research and Public Health. 2020; 17(11): 3930.
8. Blüher M. Obesity: global epidemiology and pathogenesis. Nature Reviews Endocrinology. 2019; 15(5): 288-298.
9. Choi J.R., Ahn S.V., Kim J.Y. et al. Comparison of various anthropometric indices for the identification of a predictor of incident hypertension: the ARIRANG study. Journal of human hypertension. 2018; 32(4): 294-300.
10. Siren R., Eriksson J.G., Vanhanen H. Waist circumference a good indicator of future risk for type 2 diabetes and cardiovascular disease. BMC public health. 2012; 12(1): 1-6.
11. Ohishi M. Hypertension with diabetes mellitus: physiology and pathology. Hypertension research. 2018; 41(6): 389-393.
12. Aggarwal P., Sinha S.K., Khanra D. et al. Comparison of original and modified Q risk 2 risk score with Framingham risk score-An Indian perspective. Indian Heart Journal. 2021. doi: 10.1016/j.ihj.2021.01.016.
13. Romanens M., Szucs T., Sudano I. et al. Agreement of PROCAM and SCORE to assess cardiovascular risk in two different low risk European populations. Preventive medicine reports. 2019; 13: 113–117.
14. Santos A.S., Rodrigues A.P.S., Rosa L.P. et al. Cardiometabolic risk factors and Framingham Risk Score in severely obese patients: Baseline data from DieTBra trial. Nutrition, Metabolism and Cardiovascular Diseases. 2020; 30(3):474-482.
15. Goff D.C., Lloyd-Jones D.M., Bennett G. et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Journal of the American College of Cardiology. 2014; 129(25): 49-73.
16. Khanna N.N., Jamthikar A.D., Gupta D. et al. Performance evaluation of 10-year ultrasound image-based stroke/cardiovascular (CV) risk calculator by comparing against ten conventional CV risk calculators: a diabetic study. Computers in biology and medicine. 2019; 105, 125-143.
17. American College of Cardiology/American Heart Association Guideline on the Assessment of Cardiovascular Risk (ASCVD Risk). 2013. http://tools.acc.org/ASCVD-Risk-Estimator-Plus/#!/calculate/estimate/.
18. Sohn C., Kim J., Bae W. The framingham risk score, diet, and inflammatory markers in Korean men with metabolic syndrome. Nutrition research and practice. 2012; 6(3): 246-253.
19. Paiter J., Oliveira G.M.M.D. Risk Prediction Systems: One for all or all for Some. International Journal of Cardiovascular Sciences. 2021; 34(1): 39-43.
20. Khera R., Valero-Elizondo J., Nasir K. Financial toxicity in atherosclerotic cardiovascular disease in the United States: current state and future directions. Journal of the American Heart Association. 2020; 9(19): doi: 10.1161/JAHA.120.017793.

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ORCID and contributionship:
Taras I. Griadil: 0000-0002-1048-0656 [A,B,C,D,E,F]
Ivan V. Chopey: 0000-0003-4626-0855 [A,B,C,D]
Ksenia I. Chubirko: 0000-0002-4379-0538 [C,D,E,F]
Mykhaylo M. Hechko: 0000-0003-2793-5044 [E,F]
Wael Rumaneh: 0000-0001-7860-9674 [C,E]

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CORRESPONDING AUTHOR
Taras I. Griadil
Uzhhorod National University
148 Sobranetska st., 88017 Uzhhorod, Ukraine
tel: +380990080218
e-mail: taras.griadil@uzhnu.edu.ua

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REVIEW ARTICLE

LOOP DIURETICS IN HEART FAILURE: EVIDENCE-BASED CHOICE

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Maryana M. Rosul, Miroslava M. Bletskan, Nataliya V. Ivano, Marina O. Korabelschykova
STATE HIGHER EDUCATIONAL ESTABLISHMENT "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE

ABSTRACT

The aim: Of the article is to conduct a comparative evaluation of the effectiveness of torasemide and furosemide in patients with heart failure.

Materials and methods: Analysis of the existing clinical trials and meta-analyzes that combine the results of the completed studies aimed at the investigation of comparative efficacy of furosemide and torasemide in patients with heart failure (HF).

Conclusions: There is enough convincing evidence to speak about the advantages of torasemide over furosemide both in terms of its pharmacological properties and taking into account the reduction of hospitalizations, functional progress and improvement in the quality of life of patients with HF. The safety profile of torasemide is more favorable, as it is associated with a reduced risk of hypokalemia compared to furosemide. The abovementioned facts favor the use of torasemide in patients with symptomatic HF, as well as the transition from furosemide to torasemide in patients with edema caused by HF, which remain uncontrolled despite receiving optimal doses of furosemide.

KEY WORDS: heart failure, torasemide, furosemide

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INTRODUCTION

Heart failure is one of the commonest diseases in the world. The main manifestations of HF are the symptoms associated with fluid retention and congestion, including shortness of breath, edema, and impaired perfusion of tissues and organs. In its turn, fluid retention, congestion and severe symptoms caused by them are associated with reduced performance and impaired quality of life, increased risk of hospitalization and adverse effects [1]. Therefore, use of diuretics, including loop diuretics, is an important component of complex therapy of patients with HF.

The current guidelines do not have a clear answer to the question of which of the modern loop diuretics has advantages when used in patients with HF. At the current stage, the question of choosing the best loop diuretic remains debatable [2, 3]. Furosemide appeared on the pharmaceutical market earlier than torasemide. As a result, experience of furosemide usage in clinical settings is much greater than the experience of torasemide. However, the principles of evidence-based medicine require that, if there is a choice in treatment, the drug with the best effect on the patient's quality of life and prognosis must be used.

THE AIM

The aim is to conduct a comparative evaluation of the effectiveness of torasemide and furosemide in patients with HF.

MATERIALS AND METHODS

Analysis of the existing clinical trials and meta-analyzes that combine the results of the completed studies aimed at the investigation of the comparative efficacy of furosemide and torasemide in patients with HF.

REVIEW AND DISCUSSION

Loop diuretics remain the most effective means of reducing the clinical symptoms and signs of HF. To date, there are numerous studies that indicate the advantages of torasemide over furosemide in the treatment of patients with HF.

In an open-label TORIC study (Torasemide in Congestive Heart Failure), torasemide has shown clear beneficial effects on prognosis and a more significant improvement in the clinical condition of patients with HF compared with other diuretics, including furosemide. The study involved 1377 patients with chronic HF (NYHA II-III). Torasemide at a dose of 10 mg / day compared with furosemide at a dose of 40 mg / day and other diuretics significantly reduced overall mortality – by 51.5%, cardiovascular death – by 59.7%, sudden death – by 65.8%. At the same time, functional improvement assessed as class reduction by New York Heart Association (NYHA) was observed in a larger number of patients receiving torasemide (45.8%) than in patients receiving furosemide or other diuretics (37.2%) (p=0.00017). There was also better tolerability of the drug (torasemide) with less probability of side effects. It significantly less often led to the development of hypokalemia than furosemide (12.9 and 17.9%, respectively, p=0.013) [4].

Another open-label randomized study of 237 patients with chronic HF (NYHA II-IV) (Müller K., 2003) has also shown a more significant clinical improvement by at least one NYHA class in patients treated with torasemide compared

to furosemide (p=0.014). In the given study, torasemide has shown significantly higher tolerability (p=0.0001): reduced restrictions in daily life (p=0.0002), the number of mictions at 3, 6 and 12 hours after taking a diuretic (p<0,001 at all time points) and urgency to urinate (p<0.0001) compared to treatment with furosemide [5].

In the study by Murray et al. (2001) involving 234 patients with chronic HF, there was a significant reduction in the frequency of rehospitalization for HF when comparing torasemide with furosemide (32% vs. 17%, p<0.01) and other cardiovascular causes (59% vs. 44%, p=0.03). Patients in the torasemide group were hospitalized for HF almost for a twice less period (106 vs. 296 days in the furosemide group, p=0.02) [6].

The results of several recently-conducted meta-analyzes studying comparative evaluation of the clinical effects from the mentioned loop diuretics in patients with HF have also shown the clinical advantages of torasemide over furosemide.

According to the analysis performed by DiNicolantonio (2012) there was a significant reduction in the overall risk of HF rehospitalization (p<0.0001), as well as a reduction in the risk of further HF rehospitalization (p=0.008) and cardiovascular events (p=0,03) in patients treated with torasemide and having at least one rehospitalization in the anamnesis. Moreover, the tendency for overall mortality reduction was also noticed in patients in the group of torasemide (p=0.54) [7].

The results of the meta-analysis by Shah et al. (2018) have shown that torasemide significantly reduced the risk of HF rehospitalization (p<0.0001) and cardiovascular events (p=0.01) compared with furosemide. The given results confirm the clinical advantage of the drug and indicate an increase in cost-effectiveness of HF therapy due to reduced costs of rehospitalization with torasemide: replacing furosemide with torasemide could save about $ 4 billion per year through reducing the risk of rehospitalization for HF by 67%. However, the analysis of the frequency of deaths and side effects has not revealed significant intergroup differences (p=0.38) [8].

The conducted meta-analysis by Kido et al. (2019) has shown that the use of torasemide in patients with decompensated HF was accompanied by a higher frequency of functional improvement by at least 1 NYHA class (45% vs. 36.1%, p<0.0004). In the torasemide group there was a less pronounced decrease in blood potassium level compared with the use of furosemide and other diuretics used in HF. The proportion of patients with low potassium level was significantly higher in the group of furosemide and other diuretics than in the group of torasemide (17.9% vs. 12.9%; p=0.013). There was no statistically significant difference in mortality rates between the torasemide and furosemide groups (p=0.99). Similarly, no statistically significant intergroup differences were found in the frequency of rehospitalizations for HF (p = 0.15) and for cardiovascular events (p=0.22) [9].

A meta-analysis by Miles et al. (2019) has confirmed the advantage of torasemide in reducing the risk of rehospitalization for HF and having functional improvement of NYHA class. However, overall mortality rates were similar between the torasemide and furosemide groups [10].

A meta-analysis by Abraham et al. has shown that the use of torasemide is associated with a much more pronounced functional improvement from NYHA III/IV to NYHA I/II (p=0.004), reduced mortality from cardiac causes compared with furosemide in patients with HF (p<0,001). The torasemide treatment results in a tendency to reduce the frequency of hospitalizations for HF compared with furosemide (p=0.07). The studied diuretics do not differ in the effect on overall mortality (p=0.65) and in the frequency of side effects in patients (p=0.48) [11].

Thus, all the results of the meta-analyzes of the main comparative studies of furosemide and torasemide in HF have shown that torasemide can significantly alleviate the course of HF and reduce the frequency of rehospitalizations for HF and cardiovascular events compared to furosemide. The safety profile of torasemide is also more favorable, as it is associated with a reduced risk of hypokalemia compared to furosemide. However, in terms of the effect of torasemide on the risk of death, the given meta-analyzes contradict the results of the already mentioned largest comparative study of torasemide TORIC (significant reduction in patient mortality along with vivid functional improvement of NYHA class). In this respect, it is especially long-awaited and relevant to obtain the results of the TRANSFORM-HF study launched in 2018, the main task of which is to determine whether there are differences between torasemide and furosemide in terms of impact on mortality from all the causes [12].

The favorable clinical effects of torasemide received in the course of the conducted research can be explained by important features of pharmacokinetics and mechanisms of action of the drug. The bioavailability of torasemide is a stable indicator, equals to 80–100% and does not depend on food intake or the presence of edema of the intestinal wall (common in HF). In its turn, the bioavailability of furosemide varies ranging between 10–90% and decreases in the case of taking the drug with food, as well as in patients with edema of the intestinal wall by approximately 30% [13]. In addition, torasemide is characterized by a faster onset (1.1 vs. 2.4 h) and a longer duration (18–24 vs. 4–6 h) of action compared to furosemide. Among other things, torasemide reduces the risk of postdiuretic rebound phenomenon in the form of water and sodium retention (due to lower likelihood of achieving subtherapeutic concentrations of the drug in blood compared with short-acting furosemide) [14]. Another advantage of the long-term effect of torasemide is the possibility to take the drug once a day (compared with the scheme 2 times a day, typical for furosemide). The smooth diuretic effect of torasemide is accompanied by a decrease in urination compared with furosemide and does not limit patients' activity, which in general increases adherence to treatment by approximately 13% [15].

Torasemide is proven to have an antialdosterone effect due to blockade of aldosterone receptors and decreased aldosterone synthesis [13, 16]. Due to the antialdosterone effect, torasemide slows down the process of fibrosis in the

myocardium and vascular wall, and also has a minimal kalliuretic effect, which reduces the risk of hypokalemia in contrast to furosemide. It is known that hypokalemia is associated with an increased risk of severe arrhythmias and worsening of the prognosis [11]. The use of drugs aimed at minimizing fibrosis can prevent heart dysfunction, reduce cardiac muscle stiffness, slow myocardial remodeling, reduce the risk of sudden death due to arrhythmias, improve cardiac function and reduce NYHA class, especially in patients with more pronounced myocardial fibrosis [17]. In the study by B. López et al. immediate-release (IR) torasemide has shown to reduce the bulk fraction of collagen and the development of fibrosis compared to furosemide. Myocardial fibrosis is the result of increased accumulation of type I collagen in the interstitium, as well as around the intramyocardial arteries and arterioles. Torasemide IR (according to endomyocardial biopsy) blocks the enzyme involved in the synthesis of molecules of this type of collagen [18]. Thus after 8 months of treatment it reduces the amount of collagen fraction in the myocardium in patients with chronic HF (NYHA II-IV) by 1.8 times. It is important to note that the diuretic activity and antifibrotic properties of torasemide are observed at different stages of treatment. Thus, antihypertensive and diuretic effects occur immediately after the initial dose, and the implementation of antifibrotic action can be observed after 6-9 months of continuous therapy [19].

At the same time, a large-scale TORAFIC study (the prolonged-release formulation of torasemide versus furosemide in patients with chronic heart failure) proves that the prolonged-release formulation of torasemide (torasemide-PR) has no effect on myocardial fibrosis. Inability of torasemide-PR to reduce myocardial fibrosis can probably be explained by the form of release and, consequently, the lower maximum concentration of the active substance in plasma, insufficient to trigger the mechanisms by which collagen synthesis is inhibited [20].

Considering the optimal choice of loop diuretic in patients with HF, we cannot disregard the new document of the European Society of Cardiology published in 2019 "The Use of Diuretics in Congestive Heart Failure – a Position Statement from the Heart Failure Association of the European Society of Cardiology." This document draws doctors' attention for the first time to the advisability of patients' transition to torasemide after an acute episode of HF while previously taking furosemide: "For patients who developed an acute heart failure episode while previously taking a loop diuretic before admission, a higher dose following discharge might need to be used. Additionally, in case that this previous loop diuretic was furosemide, a switch to either bumetanide or torsemide might be considered, as they have a more predictable absorption pattern and bioavailability, especially in the face of subclinical congestion" [21].

CONCLUSIONS

To date, there have been enough convincing evidence to speak about the advantages of torasemide over furosemide both in terms of its pharmacological properties and the reduction in the frequency of hospitalizations, functional improvement, improving the quality of life of patients with heart failure. The safety profile of torasemide is also more favorable, as it is associated with a reduced risk of hypokalemia compared to furosemide. All these facts favor the use of torasemide in patients with symptomatic heart failure, as well as the transition from furosemide to torasemide in patients with edema caused by heart failure, which remains uncontrolled despite receiving optimal doses of furosemide.

REFERENCES

1. Gheorghiade M., Follath F., Ponikowski P. et al. Assessing and grading congestion in acute heart failure: a scientific statement from the Acute Heart Failure Committee of the Heart Failure Association of the European Society of Cardiology and endorsed by the European Society of Intensive Care Medicine. Eur J Heart Fail. 2010; 12(5): 423-433.
2. Ponikowski P., Voors A., Anker S. et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail. 2016; 18(8): 891-975.
3. Yancy C., Jessup M., Bozkurt B. et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation. American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2013; 62(16): e147-239.
4. Cosín J., Díez J. TORIC investigators. Torasemide in chronic heart failure: results of the TORIC study. Eur J Heart Fail. 2002; 4(4): 507–513.
5. Müller K., Gamba G., Jaquet F., Hess B. Torasemide vs. furosemide in primary care patients with chronic heart failure NYHA II to IV – efficacy and quality of life. Eur J Heart Fail. 2003; 5(6):793–801.
6. Murray M.D., Deer M.M., Ferguson J.A. et al. Open-label randomized trial of torsemide compared with furosemide therapy for patients with heart failure. Am J Med. 2001; 111(7): 513-520.
7. DiNicolantonio J.J. Should torsemide be the loop diuretic of choice in systolic heart failure? Future Cardiol. 2012; 8 (5): 707-728.
8. Shah P., Patel H., Mithawala P., Doshi R. Torsemide versus furosemide in heart failure patients: A meta-analysis of randomized controlled trials. Eur J Intern Med. 2018; 57: e38-e40.
9. Kido K., Shimizu M., Hashiguchi M. Comparing torsemide versus furosemide in patients with heart failure: A meta-analysis. J Am Pharm Assoc. 2019; 59(3): 432-438.
10. Miles J., Hanumanthu B., Patel K. et al. Torsemide versus furosemide and intermediate-term outcomes in patients with heart failure: an updated meta-analysis. J Cardiovasc Med (Hagerstown). 2019; 20(6): 379-388.
11. Abraham B., Megaly M., Sous M. et al. Meta-Analysis Comparing Torsemide Versus Furosemide in Patients With Heart Failure. Am J Cardiol. 2020; 125(1): 92-99.
12. TRANSFORM-HF: ToRsemide compArisoN With furoSemide FOR Management of Heart Failure (TRANSFORM-HF). https://clinical-trials.gov/ct2/show/NCT03296813.
13. Buggey J., Mentz R., Pitt B. et al. A reappraisal of loop diuretic choice in heart failure patients. Am Heart J. 2015; 169 (3): 323-333.
14. Vargo D.L., Kramer W.G., Black P.K. et al. Bioavailability, pharmacokinetics, and pharmacodynamics of torsemide and furosemide in patients with congestive heart failure. Clin Pharmacol Ther. 1995; 57(6): 601-609.
15. Claxton A., Cramer J., Pierce C. A systematic review of the associations between dose regimens and medication compliance. Clin Ther. 2001; 23(8): 1296-1310.

16. Tsutamoto T., Sakai H., Wada A. et al. Torasemide inhibits transcardiac extraction of aldosterone in patients with congestive heart failure. J Am Coll Cardiol. 2004. 44(11): 2252-2253.
17. González A., Schelbert E., Díez J., Butler J. Myocardial Interstitial Fibrosis in Heart Failure: Biological and Translational Perspectives. J Am Coll Cardiol. 2018; 71(15): 1696-1706.
18. López B., González A., Beaumont J. et al. Identification of a potential cardiac antifibrotic mechanism of torasemide in patients with chronic heart failure. J Am Coll Cardiol. 2007; 50(9): 859–867.
19. López B., Querejeta R., González A. et al. Effects of loop diuretics on myocardial fibrosis and collagen type I turnover in chronic heart failure. J Am Coll Cardiol. 2004; 43(11): 2028-2035.
20. TORAFIC Investigators Group. Effects of prolonged-release torasemide versus furosemide on myocardial fibrosis in hypertensive patients with chronic heart failure: a randomized, blinded-end point, active-controlled study. Clin Ther. 2011; 33(9): 1204-1213.
21. Mullens W., Damman K., Harjola V. et al. The use of diuretics in heart failure with congestion – a position statement from the Heart Failure Association of the European Society of Cardiology. Eur J Heart Fail. 2019; 21(2): 137-155.

ORCID and contributionship:
Maryana M. Rosul: 0000-0002-2106-5386 [A,B,D,E]
Miroslava M. Bletskan: 0000-0002-8069-6145 [A,B,D]
Natalia V. Ivanio: 0000-0003-0147-2176 [B,F]
Marina O. Korabelschykova: 0000-0002-7632-4322 [B,F]

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CORRESPONDING AUTHOR
Maryana M. Rosul
Uzhhorod national university
148 Sobranetska Str., 88000 Uzhhorod, Ukraine
tel: +380506115733
e-mail: maryana.rosul@uzhnu.edu.ua

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REVIEW ARTICLE

NON-ALCOHOLIC FATTY LIVER DISEASE IN THE CONTEXT OF ALTERED GUT MICROBIOTA

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Tetyana V. Koval, Ivan V. Chopey, Mykhaylo M. Hechko, Artur V. Kurakh
STATE HIGHER EDUCATIONAL INSTITUTION "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE

ABSTRACT

The aim: To analyze the relationship between non-alcoholic fatty liver disease and changes in the gut microbiota.

Materials and methods: The publications of domestic and foreign editions in the databases of the United European Gastroenterology (UEG) Journal, PubMed, MEDLINE, Web of Science were processed and analyzed.

Conclusions: In recent years, non-alcoholic fatty liver disease was placed among the important diseases in gastroenterology. During this time, more and more data appear on the link between changes in the human intestinal microbiome and the development of metabolic diseases, including NAFLD. Contemporary research has indeed found evidence of such a relationship. Thus, some strains of microorganisms have been identified in more detail, which directly or indirectly affect the development or course of the above-mentioned disease. For a better understanding of the strategies for the treatment of pathologies, it is necessary to delve into the study of etiological factors, therefore, NAFLC cannot be considered a pathology that has been sufficiently studied. Indeed, recent data indicate that the development and severity of the course of the disease are not always associated with the physiological processes already known to us.

KEY WORDS: Non-alcoholic fatty liver disease, intestinal microbiota, human microbiome

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INTRODUCTION

Currently, non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide, even in developed countries, and the number of patients is constantly growing. The worldwide prevalence of NAFLC is reported to be 24% [1]. NAFLD is very common in South America and the Middle East, followed by Asia, the United States and Europe (31%, 32%, 27%, 24% and 23%, respectively), while there are fewer NAFLD patients in Africa (14 %). This pathology often has similar symptoms with other metabolic disorders, including type 2 diabetes and obesity. Recent studies have highlighted the role of gut microbiota composition in the pathophysiology of many diseases. With regard to NAFLD, experiments carried out using gut microbiota transplantation in animal models that did not have a tendency for NAFLD, showed that the development of fatty liver disease is associated with intestinal bacteria. Moreover, in patients with NAFLD, changes in the composition of the intestinal microbiota were observed.

THE AIM

Analyze the relationship between non-alcoholic fatty liver disease and changes in the intestinal microbiota based on the studies and works already described.

MATERIALS AND METHODS

To achieve this goal, publications of domestic and foreign publications found by stated keywords "Non-alcoholic liver disease", "changes in the intestinal microbiota", "overweight", "obesity" in the databases of the United European Gastroenterology (UEG) Journal, PubMed, MEDLINE, Web of Science were processed and analyzed.

REVIEW AND DISCUSSION

The microbiome of the gastrointestinal tract is represented by different bacteria, both quantity and quality. Their highest level and diversity are presented in the colon. Dominant groups of bacteria were presented by *Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes*. High levels of the latter correlated with higher individual susceptibility to pathologic conditions, including obesity. [2] However, to talk about the similarities in microbiota compositions at the population level is difficult and incorrect. After all, even identical twins have different microbiomes. [3] It is known that in the large intestine many bacteria coexist, including such important for its proper functioning bacteria as *Escherichia coli (E. coli), Campylobacter jejuni, Salmonella enterica, Vibrio cholerae and Bacteroides fragilis*, usually in very low quantities (<0, 1% intestinal microbiome). [3,4] The microbiome differs in the same parts of the intestine,

depending on its proximity to the intestinal mucosa. Thus, Bacteroides, Streptococcus, Bifdobacterium, Enterobacteriaceae, Enterococcus, Clostridium, Ruminococcus and Lactobacillus inhabit the parts that are farther from the mucosa, while Clostridium, Lactobacillus, Enterococcus and Akkermansia are located closer to its surface. [5] The composition of the microbiome is influenced by many external factors, such as diet, birth method, geographical location, genetics, etc. [6,7] The composition of the microbiome is unique to each person, but changes may occur during a lifetime and can affect the immune, metabolic and phenotypic processes of the host. [8] As stated by such authors as Kovacs and others, we it can be stated that genetics play a key role in the formation of the intestinal microbiome, as shown by studies on mice. [9] However, human subject research provide different results and so, even in genetically related individuals the structure of the intestinal microbiota didn`t have much similarities. [10,11] Various manipulations that affect the change of intestinal microbiota lead to worsening or improvement of the course of a disease, and sometimes – to development of new diseases in a person. It is believed that the the link between the biological processes of the intestinal microbiota and the development of liver diseases is observed from the moment of fetal development, because the formation of the liver begins directly from the foregut. This link remains after birth, as the liver receives 75% of the blood flow from the intestinal portal. [12] The connection between the human microbiome and the development of NAFLD was first proposed in 1980, when patients with concomitant NAFLD underwent surgical bypass of the intestine, followed by the prescription of antibacterial drugs. Antibacterial therapy improved the course of NAFLD . [13]

Different studies on humans and animals, clearly outlined the connection between dysbacteriosis and NAFLD [14,15], as well as the severity of the latter. [16] According to Spencer et al. it was found that along with the decrease in choline levels, the accumulation of fat in the liver depended on the level of Erysipelotrichia and Gammaproteobacteria in the intestine. Thus, according to these data, a higher level of Erysipelotrichia was observed in patients with a higher level of NAFLD, and a higher level of Gammaproteobacteria – on the contrary, was found in patients with a low NAFLD development. Comparisons were made between the two groups – underweight and obese patients. It was found that patients with NAFLD had an increase in the level of gram-negative bacteria (Bacteroidetes up to 20%) and a decrease in the level of Firmicutes (SCFA-producing Lachnospiraceae, Lactobacillaceae and 7α-dehydroxylating Ruminococcaceae) to 24% compared to patients without obesity. However, in patients with NAFLD, there was an increase in the number of opportunistic pathogenic microorganisms that produce lipopolysaccharides, compared to the other group of patients. [17]

In 2017, new data emerged stating that even different forms of nonalcoholic fatty hepatosis are accompanied by different balance ratios of the intestinal microbiota. Thus, according to the Loomba et al, in patients with mild to moderate severity forms of NAFLD, a higher level of Firmicutes was observed compared to patients with severe fibrosis. In regards to Proteobacteria the situation was different – higher levels were found in patients with severe forms of fibrosis. In turn, Eubacterium was observed in higher numbers in patients with mild forms of fibrosis, while elevated levels of E. coli were detected in later stages of NAFLD, however, in this group of patients there was a decreased level of Ruminococcus obeum and E. Rectale [18] There was also other evidence of a relationship between intestinal microbiota imbalance and the development of NAFLD. Studies involving other classes of the intestinal microbiota, such as Allisonella and Parabacteroides, as well as Anaerosporobacter and Faecalibacterium, have shown that the former were more common in patients with a concomitant diagnosis of NAFLD than the other three [19].

According to Mouzaki et al. lower levels of Bacteroidetes were also observed in patients with nonalcoholic hepatosis, compared to patients without fibrosis. Moreover, according to these data, significant differences in the composition of the intestinal microbiota between patients with mild fibrosis and healthy patients were not found. [14]

Despite data on the influence of genetic factors on the composition of the hosts` intestinal microbiota, it is known that changes in the relation of different species of bacteria also depend on lifestyle, including malnutrition, urbanization, reduced physical activity, worsened living conditions, uncontrolled use of antibiotics and improved hygiene. All of these factors affected the composition of our microbiota, as well as the emergence of so-called diseases of modern civilization, which contributes to changes in the microbiota and morbidity. [20] Diet is considered to be one of the most powerful aspects of microbial communities [21,22]. Studies have shown, that such changes in diet, like inclusion of high levels of sugar and fat, deeply affected the gut microbiota, with changes in the relation of Firmicutes to Bacteroidetes and an abrupt decrease in bacterial variety[23,24]

Recent data show that the appointment of antibacterial drugs, as the main method of treatment of many diseases today, leads to a significant reduction of important for the proper functioning of the body, microorganisms, and sometimes their disappearance from the patient's microbiome. [25] Instead, the literature describes data on the positive effects of prescribing antibacterial drugs, in particular in the treatment of NAFLD. Models were described, in which treatment with antibacterial drugs lasting 6 months, mainly the subsequent use of norfloxacin and neomycin, led to a decrease in bacterial growth of the microbiota in the intestinal lumen, which improved the course of NAFLD, as well as improved liver function in cirrhosis. [26] According to Gangarapu V et al., administration of rifaximin may reduce circulating endotoxin (LPS) and alanine aminotransferase levels in patients with NAFLD. [30] Back in 2008, Wu W showed that experimental oral administration of cidomycin increased the rate of transit through the small intestine and decreased the serum alanine aminotransferase, aspartate aminotransferase and

tumor necrosis factor-α levels in rats with NASH, indicating the cydamicins potential for lowering NASH activity by changing the gut microbiome. [27]

However, recent reports show an increasing trend towards antibiotic resistance, so the widespread use of antibiotics is currently accompanied by a highly negative health response.

In recent years, there have been reports of a positive effect from intensive growth of some microorganisms on human health, namely, it was found that Akkermansia muciniphila is involved in the destruction of mucin, which, in turn, is positively correlated with weight loss and reduction of NAFLD risk. [28]

According to the latest EASL / EASD / EASO recommendations, pharmacological treatment of NAFLD begins only when liver fibrosis is confirmed. In other cases, non-pharmacologic methods are use, which mainly consist of lifestyle changes. However, the recommendations do not include any treatment aimed at correcting the composition of the intestinal microbiota, as this issue is not sufficiently studied.

Despite all the factors, at the moment diet remains one of the universal methods of influencing the composition of the microbiota. It is known that the entire human microbiome is actively involved in digestion. Its main role is to breakdown and absorb substances from food. Substances that penetrate the epithelial barrier, affect even the most remote cells of the body, using the bloodstream as a method of transportation. [29] This is supported by descriptions of L-carnitine metabolism, which is contained mainly in red meat. Gut microorganisms and the liver convert it into trimethylanin N-oxide, which leads to the development of atherosclerosis. Thus, according to RA Koeth, in vegetarians the composition of the microbiota contributes to significantly less formation of trimethylanin N-oxide compared to those who include meat in the diet. [30]

CONCLUSIONS

Thus, the intestinal microbiota may be associated with detrimental effects on the liver, in particular, the development of NAFLD, and changing the composition of the intestinal microbiota through diet may be an effective strategy to improve the course of liver disease. The main goals of steatohepatitis treatment include the reduction of its stage, lowering insulin resistance, prevention of steatosis progression and reduction of oxidative stress. The cornerstone of NAFLD treatment, of course, is lifestyle changes. At the same time, adherence to low calorie diet and reduction of consumption of foods and beverages with high fructose content, along with weight loss and reduction of liver steatosis, may quickly change the intestinal microbiome. The purpose of diet therapy is a gradual decrease in body weight by 1.5-2 kg per month (up to 7% or more) – this may reverse hepatic steatosis. In NASH, however, weight loss should be ≥10% of baseline.

Based on the above, there is an urgent need for further research to identify the link between the intestinal microbiota and the development and progression of NAFLD.

REFERENCES

1. Younossi Z.M., Koenig A.B., Abdelatif D. et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology (Baltimore, MD). 2016;64(1):73–84.
2. Ley R.E., Turnbaugh P.J., Klein S., Gordon J.I. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022–1023.
3. Huttenhower C., Gevers D., Knight R. et al. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–214. https ://doi.org/10.1038/nature11234.
4. Gillespie J.J., Wattam A.R., Cammer S.A. et al. PATRIC: the comprehensive bacterial bioinformatics resource with a focus on human pathogenic species. Infect Immun. 2011;79(11):4286–4298.
5. Swidsinski A., Loening-Baucke V., Lochs H., Hale L.P. Spatial organization of bacterial fora in normal and infamed intestine: a fuorescence in situ hybridization study in mice. World J Gastroenterol. 2005;11(8):1131–1140.
6. Brown C.T., Sharon I., Thomas B.C. et al. Genome resolved analysis of a premature infant gut microbial community reveals a Varibaculum cambriense genome and a shift towards fermentation-based metabolism during the third week of life. Microbiome. 2013;1(1):30. https ://doi.org/10.1186/2049-2618-1-30.
7. Clemente J.C., Ursell L.K., Parfrey L.W., Knight R. The impact of the gut microbiota on human health: an integrative view. Cell. 2012;148(6):1258–1270.
8. Macpherson A.J., de Aguero M.G., Ganal-Vonarburg S.C. How nutrition and the maternal microbiota shape the neonatal immune system. Nat Rev Immunol. 2017;17(8):508–517.
9. Kovacs A., Ben-Jacob N., Tayem H. et al. Genotype is a stronger determinant than sex of the mouse gut microbiota. Microb Ecol. 2011;61(2):423–428.
10. Turnbaugh P.J., Ridaura V.K., Faith J.J. et al. The efect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med. 2009;1(6):6-14. https://doi.org/10.1126/scitranslm ed.3000322 .
11. Yatsunenko T., Rey F.E., Manary M.J. et al. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222–227.
12. Sleisenger M.H., Feldman M., Friedman L.S., Brandt L.J. Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management, 9th ed. Philadelphia, PA: Saunders/Elsevier. 2010.
13. Drenick E.J., Fisler J., Johnson D. Hepatic steatosis after intestinal bypass–prevention and reversal by metronidazole, irrespective of protein-calorie malnutrition. Gastroenterology. 1982;82(3):535–548.
14. Mouzaki M., Comelli E.M., Arendt B.M. et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology (Baltimore, MD). 2013;58(1):120–127.
15. Zhu L., Baker S.S., Gill C. et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology (Baltimore, MD). 2013;57(2):601–609.
16. Yari Z., Rahimlou M., Eslamparast T. et al. Flaxseed supplementation in non-alcoholic fatty liver disease: a pilot randomized, open labeled, controlled study. Int J Food Sci Nutr. 2016;67(4):461–469.
17. Wang B., Jiang X., Cao M. et al. Altered fecal microbiota correlates with liver biochemistry in nonobese patients with non-alcoholic fatty liver disease. Sci Rep. 2016;6:32002.
18. Loomba R., Seguritan V., Li W. et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab. 2017;25(5):1054–1062. e1055.

19. Wong V.W., Tse C.H., Lam T.T. et al. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis—a longitudinal study. PLoS One. 2013;8(4):e62885.
20. Sleisenger M.H., Feldman M., Friedman L.S., Brandt L.J. Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology, Diagnosis, Management, 9th ed. Philadelphia, PA: Saunders/Elsevier. 2010.
21. David L.A., Maurice C.F., Carmody, R.N. et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505:559–563. doi:10.1038/nature12820.
22. Muegge B.D., Kuczynski J., Knights D. et al. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science. 2011;332: 970–974. doi:10.1126/science.1198719.
23. Shankar V., Gouda M., Moncivaiz J. et al. Differences in gut metabolites and microbial composition and functions between Egyptian and U.S. children are consistent with their diets. mSystems. 2017; 2: e00169-16.
24. Voigt R.M., Summa K.C., Forsyth C.B. et al. The circadian clock mutation promotes intestinal dysbiosis. Alcohol.-Clin. Exp. Res. 2016;40: 335–347.
25. Dethlefsen L., Huse S., Sogin M.L., Relman D.A. The pervasive efects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008;6(11):e280.
26. Madrid A.M., Hurtado C., Venegas M. et al. Long-Term treatment with cisapride and antibiotics in liver cirrhosis: efect on small intestinal motility, bacterial overgrowth, and liver function. Am J Gastroenterol. 2001;96(4):1251–1255.
27. Wu W. Small intestinal bacteria overgrowth decreases small intestinal motility in the NASH rats. World J. Gastroenterol. 2008;14: 313.
28. Dao M.C., Everard A., Aron-Wisnewsky J. et al. Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut. 2016;65: 426–436. DOI 10.1136/gutjnl-2014-308778.
29. Ridaura V.K., Faith J.J., Rey F.E. et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013; 341 (6150): 1241214. DOI 10.1126/science.1241214.
30. Koeth R.A., Wang Z., Levison B.S. et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med. 2013;19:576–585. DOI 10.1038/nm.3145.

ORCID and contributionship:
Tetyana V. Koval: 0000-0001-8904-041X [A,B,D]
Ivan V. Chopey: 0000-0003-4626-0855 [E,F]
Mykhaylo M. Hechko: 0000-0003-2793-5044 [A,B]
Artur V. Kurakh 0000-0002-2763-2935 [B,E]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Tetyana V. Koval
Uzhhorod National University
77 Minayska St., 88000 Uzhhorod, Ukraine
tel: +3806664382879
e-mail: t.duxowi41992@gmail.com

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REVIEW ARTICLE

SHORT- AND LONG-TERM EFFECTS OF NSAIDS ON THE GASTROINTESTINAL MUCOSA: COMPLEX ANALYSIS OF BENEFITS AND COMPLICATIONS PREVENTION

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Yana Y. Hnepa, Ivan V. Chopey, Ksenia I. Chubirko, Andriy M. Bratasyuk
STATE HIGHER EDUCATIONAL ESTABLISHMENT «UZHHOROD NATIONAL UNIVERSITY», UZHHOROD, UKRAINE

ABSTRACT

The aim: To analyse data from recent studies, dedicated to the use of non-steroidal anti-inflammatory drugs (NSAIDs); to evaluate the best clinical practice in the use of NSAIDs in order to prevent side effects (SEs) in different clinical scenarios; to optimise treatment of patients at risk of NSAIDs-related SEs.

Materials and methods: A comprehensive bibliographic search was performed using the keywords "NSAIDs", "NSAID gastropathy", "NSAID enteropathy", "complications of NSAID therapy", "cardiovascular disease", "cardiovascular risk" in the PubMed, Web of Science, Cochrane Library, Google Academy databases.

Conclusions: NSAID-induced gastrointestinal lesions are a relevant problem of internal medicine, this is due to the fact that the pathogenic mechanisms of this process are still unclear. All the gastrointestinal tract (GIT) related risk factors(RFs) for gastro- and enterocolonopathies associated with the use of NSAIDs should be taken into consideration by physicians of all specialties. The examination and diagnostic of the GIT should be performed regularly to prevent complications. Uncontrolled, long-lasting, unprescribed NSAID usage should draw the attention of doctors, especially in patients with comorbid states.

KEY WORDS: non-steroidal anti-inflammatory drugs; NSAID gastropathy; NSAID enteropathy; complications of NSAID therapy; cardiovascular diseases; cardiovascular risk; diclofenac; nimesulide; celecoxib; complications prevention

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INTRODUCTION

Literature data from many recent studies indicate a significant increase in the incidence of drug-related gastrointestinal tract (GIT) lesions. The topic is of paramount importance in clinical practice [1]. Drugs that have adverse effects on the GIT include antibiotics, aspirin and other anti-platelets drugs, steroids, anti-hypertensives, and other agents. However, most of GIT lesions are associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs), primarily during uncontrolled usage. The duration, frequency and dosage of NSAID prescriptions are quite diverse and physicians across all specialties use them in their daily practice, including therapists, pediatricians, cardiologists, rheumatologists, but most often – general practitioners – family medicine. Worldwide, several million people of all ages use NSAIDs daily as prescribed by their doctor [2]. In the United States and developed countries of Europe, 70% of people older than 65 take NSAIDs at least once a week, and 34% of them take NSAIDs every day, despite the presence of risk factors, the development of undesirable adverse effects, in the GIT and cardiovascular system (CVS), kidneys and liver [3].Such statistics are mainly explained by the uncontrolled use of these drugs which are sold over-the-counter. Compared to the usage of NSAIDs when prescribed by a doctor, self-prescribed usage occurs 7 times more often. Patients often cannot evaluate the potential interactions that might occur between NSAIDs and other medication, used by the patient to treat other conditions that they might have. Unfortunately, such uncontrolled behavior can considered as an additional RFs for the development of SEs, such as dyspepsia, peptic ulcer formation, bleeding, obstruction, and perforation. NSAIDs can damage the GIT from the esophagus to the rectum, but the proximal section is about 6 times more likely to be affected. The most common and potentially dangerous SE is the so called NSAID-induced gastropathy. This condition manifests with erosive and/or ulcerative lesions of the gastroduodenal tract, which are seen during endoscopy [4,5] in the form of acute multiple gastric erosions and/or ulcers in the absence of local inflammation and histological changes [6].

THE AIM

The aim was to analyze data from recent studies dedicated to the use of non-steroidal anti-inflammatory drugs (NSAIDs); to evaluate the best clinical practice in the use of NSAIDs in order to prevent side effects (SEs) in different clinical scenarios; to optimize treatment of patients at risk of NSAIDs-related SEs.

MATERIALS AND METHODS

We identified and reviewed a total of 130 publications published between 2006 and 2021.

REVIEW AND DISCUSSION

The increase in the number of cases and severity of intestinal lesions (NSAID-induced enteropathies and colopathy), which are much more difficult to diagnose, is unreasonably overlooked. They account for 40% of all NSAID-related complications. However, lesions of the GIT mucosa do not occur in all patients taking NSAIDs and are often associated with additional RFs, including: age over 65 year; co morbid states (e.g., cardiovascular or kidney disease); known history GIT lesions and/or GIT complications; the use of high doses of NSAIDs, as well as a combination of two or more NSAIDs; concomitant use of low doses of aspirin; concomitant use of anticoagulants and glucocorticosteroids; Helicobacter pylori (HP) infection.

According to the recommendation of the American College of Gastroenterology (ACG), additional RFs include: taking NSAIDs on an empty stomach (before eating); unhealthy lifestyle, which include smoking, abusive drinking and concomitant cardiovascular, liver and renal diseases [7].

Depending on the presence of several RFs, patients can be stratified for the risk of NSAID-induced GIT lesions into the following risk groups: high risk (recent complications of peptic ulcer disease in the anamnesis, as well as 3 RFs or more); moderate risk (1-2 RFs); low risk (no RFs) [8, 9].

It is recommended to avoid or limit the administration of NSAIDs to subjects with high cardiovascular risk (CVR), on the SCORE scale (≥10%), especially in the presence of myocardial infarction (MI), acute coronary syndrome, clinically severe coronary heart disease, aortic aneurysm, acute cerebrovascular disease, progressive chronic heart failure (CHF) and other significant cardiovascular comorbidities. Particular caution should be also exercised in patients affected by diabetes mellitus (DM) with end-organ damage, or other RFs, such as chronic kidney disease, especially when the glomerular filtration rate <30 ml/min/1,73 m^2(you may have mixed two sentences, revise) [10].The results of a multicenter cross-sectional observational study of patients with osteoarthritis (OA) conducted in Spain showed that among 17,000 patients with OA, 60.3% of patients had a high gastrointestinal risk, while 32% had a history of cardiovascular events and uncontrolled arterial hypertension (AH) was recorded in 22.6%. And despite having RFs and contraindications, to more than 50% of those patients, NSAIDs were prescribed for analgesia [11]. Therefore, the question of safe and effective NSAIDs prescription is still relevant, taking into account all RFs and comorbidities [12].

The basis for adequate administration of this class of drugs is a correct understanding of their action, the mechanism of pathogenesis, and the possible development of GIT complications. NSAIDs have been shown to inhibit prostaglandin (PG) synthesis from arachidonic acid by blocking the enzyme cyclooxygenase (COX) [13]. To date, two isoforms of COX are known: COX-1, located in the cells of various organs, provides their normal function by regulating the production of PGs.The isoform COX-2 is formed when various inflammatory processes occur in the body and provide the synthesis of pro-inflammatory PGs. The anti-inflammatory effect of NSAIDs is associated with the blockade of COX-2, while the SEs on the GIT, kidneys, CVS, liver are mediated by COX-1 inhibition. Recently, data of the new isoform COX-3, have emerged, whose inhibition leads to a decrease in the content of PG E2. Since the latter is found in the cerebral cortex and heart, it provides a central antipyretic and analgesic effects [14]. The basis of the mechanism of action for all NSAIDs is the suppression of both COX-1 and COX-2. The selectivity of NSAIDs is usually considered in the context of the predominant inhibition of COX-2, but the selectivity index of COX-2 is defined as the ratio of inhibitory activity of COX-1 / COX-2. Thus, a lower selectivity index provides more inhibition of COX-2 by NSAIDs. [6, 15].

The basis of the pathological changes in the gastrointestinal tract is the disruption of three levels of protection of the mucous membrane (MM): chemical (muco-bicarbonate), cellular, and tissue [16, 17]. The imbalance between protective and damaging factors underlies ulcerogenesis [18-20]. Several theories aim at explaining the development of GIT pathologies. The first of them is vascular, the main idea of which is ischemia of the gastric wall as a RF of ulceration. According to the peptic theory, pepsin and hydrochloric acid (HCl) are the main damaging factors affecting the gastric mucosa. ("without acid, there is no ulcer") The inflammatory theory identifies the gastritis as a pre-ulcerative condition. Another theory is the so called neurovegetative, according to which pathological changes of the mucous membrane are secondary to dysfunction of the autonomic nervous system. The theory of stress explains the relationship between the formation of ulcers and the production of mucus and its components. In addition to these theories, there is evidence supporting the role of HP in the pathogenesis of peptic ulcer disease. However, most of these theories mainly explain the development of gastropathy and do not fully cover the pathogenesis of ulcerative lesions of the intestine [21-24].

According to authors Bjarnason I and Takeuchi K, the total number of lesions of the distal intestine on the background of NSAIDs may even exceed the number of cases detected in the upper tract [25-26]. To better understand the effects of NSAIDs, namely ulcerogenesis, several pathogenetic mechanisms need to be considered. According to the first, the increase in the synthesis of leukotrienes and peptide-leukotrienes due to the metabolism of arachidonic acid leads to the development of infiltration of the MM by neutrophils [6, 27].

The next mechanism is the activation of lipid oxidation and as a consequence the accumulation of free radicals in tissues. NSAIDs are thought to disrupt the glutathione reduction cycle, which plays an important role in neutralizing free radicals [27-29].

The most studied is the third mechanism of damage to the intestinal MM, which describes the ability of NSAIDs to inhibit the synthesis of nitric oxide (NO), derivatives of which (peroxynitrite) have a local altering effect on MM [30].

Table 1. Classification of nonsteroidal anti-inflammatory drugs depending on their ability in therapeutic doses to selectively block the activity of COX-1 and COX-2 (J. Frolich, 1997)

Group of drugs	Preparations
Selective COX-1 inhibitors	Low doses of acetylsalicylic acid
Non-selective COX-1 and COX-2 inhibitors	Diclofenac, ibuprofen, piroxicam, indomethacin, naproxenandothers
Selective COX-2 inhibitors	Meloxicam, nimesulide
Highlyselective (specific) COX-2 inhibitors	Celecoxib, rofecoxib

Note: COX iscyclooxygenase.

The mechanisms of development of gastro- and endo-colonopathy differ. After all, enteropathy develops not only as a result of local NSAIDs effect, but also due to their systemic action.Like gastropathy, enteropathies develop because of inhibition of COX-1 and COX-2 activity, which contributes to tissue damage. The basis of NSAIDs-associated enteropathies lies in the cumulative effects on the intestine. The above drugs have the ability to be absorbed in the ileum and by enterohepatic recirculation to be released into the duodenum together with bile, which leads to even greater damage to the mucous membrane. Damage of the intestine occurs due to rupture of the lipid bilayer of epithelial cells by separation of oxidative phosphorylation. Whereas, in the development of NSAIDs gastropathy the critical moment is the adhesion of leukocytes to vascular endothelium [22, 31-34].The issue of changing the quantitative and qualitative composition of the microflora against the background of the use of NSAIDs deserves special attention. Physiologically normal microflora protects the MM of the intestine from aggressive factors, and long-term use of NSAIDs leads to an increase in the content of gram-negative bacteria and its detrimental effect, which has been confirmed in several studies [22, 31]. Excess bacterial growth in the small intestine can lead to suppression of gastric secretion. Moreover, the use of proton pump inhibitors (PPIs) can worsen the course of NSAID enteropathy [22, 31].

Deepening knowledge and accumulation of clinical data on the mechanisms of action of NSAIDs allowed to form a working classification and divide all existing NSAIDs into four groups (Table I).

To understand, which drugs of this groups, should be prescribed, let's look through the most commonly used NSAIDs in Ukraine. We will analise their safety and rates of development of most common complications associated with their use, taking into account the individual sensitivity and comorbidities of the patient.

Acetylsalicylic acid (ASA), as a representative of selective COX-1 inhibitors and a drug that is most often used in clinical practice by physicians all around the world and particularly in Ukraine. In cardiology, ASA is successfully used for secondary prevention of cardiovascular disease (CVD), in the form of mono and combination therapy. The frequency of ASA use, as a representative of NSAIDs, forces us to focus on a wide range of SE in the GIT. The proximal parts of the GIT are most often affected. Studies have shown that the frequency of occurrence of esophageal erosions is around 22%, of ulcers – 0.9%, erosions of the stomach and / or duodenum – 63.1%, and gastric and / or duodenal ulcers – 40% [35, 36].

According to Srinivasan A. and De Cruz P. the ulcerogenic effect of ASA on the intestine may be greater than on the stomach and duodenum. According to current data, the incidence of enteropathy associated with ASA is 50-71%, which is due to continuous lifelong use by cardiac patients [37].

In 1986 I. Bjarnason and co-authors published the results of their study, which studied the effects of NSAIDs, including ASA, ibuprofen, and indomethacin on the intestine. According to the data obtained, even short-term (within 7 days) use of ASA led to a damage of the barrier function of the intestine, and long-term to inflammation of the small intestine.

Additionally, it was found that the formation of ulcers, strictures, bleeding, as well as exacerbation and complications of inflammatory bowel disease, were caused by NSAIDs, in particular ASA [38, 39].

According to the World Health Organization, even short-term use of prophylactic doses of aspirin or a single loading dose can increase the risk of serious GIT complications in the form of subepithelial hemorrhages and erosions. This , in turn, prompted the search for ways to prevent GIT lesions which occur during ASA use. According to the CURE study there may be clear linear relationship between the dose of this drug and the ulcerogenic effect on the GIT. The study results show that the frequency of GIT bleeding directly depends on the dose of ASA: at a dose of less than 100 mg/day, bleeding was observed in 1.2% of patients; at a dose of from 100 to 200 mg/day – in 1.7%; and at a dose of ASA over 200 mg/day – in 2.5% of patients [40].

Therefore, when conducting long-term antiplatelet therapy, the minimum effective dose of ASA is usually recommended.

Nowadays, intestinal-soluble forms of ASA are increasingly used, but they are not completely safe, as they can affect the MM of the small intestine and lead to the development of multiple petechiae, erosions, and ulcers.

The results of the data obtained in the study showed better tolerability of ASA in the enteric form but risk of development to severe GIT complications was not affected [41].

W. Kubler, H. Dariuss howed that the incidence of complications did not depend on the form of ASA, which was accompanied by a higher incidence of GIT bleeding and perforation in patients receiving enteric ASA compared with the traditional form [42].

According to Henry D. et al., the pathogenesis of GIT complications is based on the systemic action of the drug. This implies that its enteric form cannot solve the problem of GIT complications development at the moment [43].

Therefore, when deciding on the appointment of ASA, health professionals need to consider the expected benefits of its prescriptions and assess the risk of hemorrhagic complications, in particular from the GIT, because the probability of complications in the first month is three times higher than regular use. If possible, avoid the simultaneous appointment of NSAIDs, glucocorticosteroids, and anticoagulants due to the increased risk of bleeding [44-46].

If NSAIDs are needed concomitantly for analgesic or antipyretic purposes, paracetamol and/or ibuprofen should be preferred. The combination of selective COX-2 inhibitors with ASA is more justified in terms of GIT MM protection, but is not justified in terms of the risk of CVS complications and requires additional gastro-protectants.

The most common analgesic taken by patients of all ages with and without a prescription is ibuprofen. The drug belongs to the group of non-selective inhibitors of COX-1 and COX-2, despite this, ibuprophen showed the lowest risk of NSAID-associated gastropathy, erosions, and ulcers of the stomach and duodenum when compared to other NSAIDs. For ibuprophen the risk was 1.0, for diclofenac it was 2.3, for aspirin – 4.8, for indomethacin – 8.0, for piroxicam – 9.0, for ketoprofen – 10.3. [47]. Analysis of the data of a cohort of several studies conducted in the United States and Canada showed similar results [48].

Ibuprofen is a relatively safe drug only when used in low, analgesic doses. Increasing the dose of this drug leads to changes in the acidity of gastric juice and the development of NSAIDs-related gastropathy and its complications [49,50].

Diclofenac has long been considered the "gold standard" of non-selective NSAIDs. This drug is one of the most studied and frequently used representatives of this group. It has a well-defined anti-inflammatory and analgesic effect, which is the key to success in patients with degenerative and rheumatic diseases. According to the large MELISSA study involving more than 9,000 patients with osteoarthritis who received meloxicam, 38% were more likely to refuse treatment because of its ineffectiveness compared with diclofenac (80 of 4635 vs. 48 of 4688; $p<0.01$) [51]. This indicates a better clinical efficacy of diclofenac compared to meloxicam. Diclofenac has a lower incidence of gastrointestinal damage compared to ibuprofen and naproxen, which is associated with predominant COX-2 inhibition. The study data was also confirmed by other population-based studies that showed a lower risk of gastrointestinal bleeding with diclofenac and ibuprofen compared with indomethacin, piroxicam, and ketoprofen. However, recent studies have shown that diclofenac is associated with an increased risk of MI, drug-induced hepatitis, and acute liver failure. This confirms the fact that this drug cannot be considered completely safe. All non-selective NSAIDs differ in the degree of adverse event (AE) on body systems. So it is necessary to use selective COX-2 inhibitors, which show a high safety profile, taking into account RFs for the development GIT lesions and comorbidities [52].

Nowadays, nimesulide is a widely used selective NSAID in Ukraine. Among the advantages of this drug are high bioavailability, rapid analgesia, powerful anti-inflammatory effect, and low frequency of side effects. Easy tissue permeability and its ability to quickly concentrate in the focus of inflammation are associated with its molecule, which has alkaline properties [53]. Nimesulide has low interference with COX-1, so that its use is associated with a lower risk of GIT complications. According to experts of the European Society of Cardiology, such as Schmidt M., Lamberts M., Olsen A.M., the drug occupies an intermediate position between meloxicam and celecoxib [54]. The expressed analgesic, anti-inflammatory action of nimesulide in various dosage forms allows its use for local and systemic inflammation. This has been confirmed by several studies.

Thus, one meta-analysis showed that among 10,608 reports describing 16 571AE due to therapy with various NSAIDs, nimesulide was twice as likely to cause GIT complications than other NSAIDs. The frequency of side effect (SE) in patients who participated in the study, was 10.4% for nimesulide group for diclofenac – 21.2%, for ketoprofen – 21.7%, for piroxicam – 18.6% [55].

Another epidemiological study by J. Laporte et al. analyzed 2,813 cases of GIT bleeding with nimesulide and some other NSAIDs. The control group consisted of 7,193 patients without GIT complications. Nimesulide was found to be safer than many other NSAIDs commonly used in Europe: the risk of bleeding was 3.2 for nimesulide, 3.7 for diclofenac, 5.7 for meloxicam, and 7.2 for rofecoxib [56].

Increasingly attention is focused on the hepatotoxicity of NSAIDs, which is quite debatable. Diclofenac sodium and aspirin are toxic to the liver. Later there were data on idiosyncratic lesions of the liver that occurred after taking indomethacin, diclofenac, sulindac. The issue of hepatotoxicity of nimesulide is increasingly discussed in foreign publications [57-59]. Some Italian scientists have conducted studies to assess the hepatotoxicity of various NSAIDs, including nimesulide, but the risk of liver damage in patients taking nimesulide and other NSAIDs, including diclofenac and ibuprofen, was not high [60].

A systematic review of randomized controlled clinical trials analyzed the use of various NSAIDs (naproxen, ibuprofen, diclofenac, celecoxib, rofecoxib, valdecoxib, meloxicam) in patients with osteoarthritis or rheumatoid arthritis. Patients that used diclofenac and rofecoxib were found to have increased level aminotransferases (3.55% and 1.8%, respectively) compared with placebo (0.29%) and other NSAIDs (0.43%). The incidence of severe hepatic impairment associated with NSAIDs was very rare and did not exceed 0.04-0.06% compared with placebo. Drug withdrawal due to hepatotoxic reactions was observed only in patients taking diclofenac (2.17%), while for other NSAIDs it was not statistically significant. Of the 37,671 hospitalized patients included in the study, only 1 case was associated with hepatotoxic effects of NSAIDs, and of the 51,942 deaths, only 1 case was associated with liver

damage in the background of NSAID treatment; both patients used naproxen [61]. Diclofenac and rofecoxib had higher aminotransferase elevations than placebo and other NSAIDs studied. None of the NSAIDs studied were found to increase the incidence of toxic liver damage, hospitalizations, or deaths.

According to the Study of Acute Liver Transplant (SALT) – a study of NSAIDs-exposed acute liver failure in European transplant centers, conducted by EMA, which involved 54 transplant centers in Europe, the rate of acute liver failure for the most common NSAIDs in these countries was: 3.3 cases per 1 million for diclofenac, 5.9 for nimesulide and 8.2 for ibuprofen [62]. A similar figure for paracetamol (except in cases of overdose) was 9.8. In two more epidemiological studies conducted in Italy, researchers Mauro Venegoni, Roberto Da Cas, etc., found that the level of all hepatopathies per 100 thousand human years for nimesulide was 35.2, for ketoprofen – 25.2, for diclofenac – 39.2, ibuprofen – 44.6 [63].

A recent systematic review and meta-analysis of 5 observational studies in South Korea showed a toxic effect of nimesulide on the hepatobiliary system [RR 2.21, 95% CI 1.72–2.83]. According to these studies, the incidence of hepatotoxicity was significantly higher in patients taking nimesulide compared with other NSAIDs. Almost half of the patients receiving nimesulide (45.5%) required a liver transplant, or died of fulminant liver failure, and a third developed hepatotoxicity within two weeks of taking nimesulide. Therefore, recent studies confirm that nimesulide, being safer for GIT complications, has an increased risk of hepatotoxicity [64].

This issue requires further studies to investigate the effect of drug dose and duration of treatment on liver damage.

This prompted the creation of a new group of NSAID – coxibs. According to the developers, these are specific COX-2 inhibitors. The first of the class of coxibs was celecoxib. The peculiarity of these drugs is their higher affinity for COX-2 rather than COX-1. It is believed that this affinity of coxibs is based on the stereometric structure of molecules, which enables non-competitive blocking of the active center of the isoenzyme [31, 34]. At the same level, there is a low dose-dependence in contrast to other NSAIDs. This specificity of COX-2 inhibitors is of interest to practitioners in the context of drug safety. According to studies, long-term use of coxibs in rheumatic diseases, much less often accompanied by the development of gastrointestinal complications [31-34]. However, based on the results of the VIGOR study, cardiovascular safety after the use of coxibs is questionable. Namely, according to the results of 62 studies, which included 94.599 patients, the incidence of MI was higher with selective NSAIDs than non-selective NSAIDs, but a significant increase in risk was observed only when taking rofecoxib. The results of the study showed that the incidence of MI with rofecoxib was higher compared with the group of patients who took naproxen for rheumatoid arthritis (RA). However, at high risk of gastrointestinal enteropathy, the use of naproxen in combination with PPIs still had higher risk of development of GIT complications, compared to coxib monotherapy. This drug was developed to ensure gastrointestinal safety, prevention of enteropathies, but the increase in cardiovascular events wasn`t taken into account.

The randomized MEDAL clinical trial also showed that etoricoxib had a negative effect on blood pressure and had almost the same number of cardiovascular complications as diclofenac [65]. The Committee for Medicinal Products for Human Use (CHMP) and the European Medicines Agency (EMEA) has concluded that the use of selective COX-2 inhibitors is associated with an increased risk of thrombotic heart disease, vascular events, such as heart attack and stroke, as well as decompensation of hypertension (AH) and increased blood pressure. Therefore, according to EMEA experts, COX-2 inhibitors should not be recommended for patients with ischemic heart disease who have suffered a stroke or who have been diagnosed with peripheral artery disease.

However, inconsistent was the fact that the number of serious complications of the proximal and distal GIT on the background of taking etoricoxib and diclofenac was equal: 0.3 and 0.32 and 0.32 and 0.38 per 100 patient-years, respectively [66, 67]. This fact wasa serious surprise, because the main advantage of coxibs, which distinguishes them from "traditional" NSAIDs, was the reduction of the risk of dangerous complications from the GIT.

An important factor influencing the development of complications from various organs and systems associated with the use of NSAIDs is their timely diagnosis. There is no specific diagnosis, but the possibility of complications from organs and systems, especially in patients at risk, encourages us to perform periodic laboratory tests, actively detectin stances HP infection, prescribe fecal testing for occult blood and calprotectin detection. The main methods of diagnosing lesions of the proximal and distal GIT areesophagogastroduodenoscopy (EGDS) and colonoscopy. If necessary, it is possible to conduct daily esophageal-impedance-pH-monitoring. Examination of the small intestine is less availablebecause video capsule endoscopy is a more technically complex, expensive, and less accessible examination than colonoscopy.

All of the factors stated above compel scientists to focuses on the development of new methods of primary prevention of the most common complications caused by taking non-steroidal anti-inflammatory drugs. In the presence of risks of gastrointestinal complications associated with NSAIDs, selective COX-2 inhibitors are preferred. Additionally, studies have found the best safety profile in both coxibs and selective NSAIDs. To prevent the development of cardiovascular complications with coxib use, it is possible to prescribe non-selective NSAIDs with concomitant use of gastroprotective agents, primarily proton pump inhibitors and/or gastroprotectors.

CONCLUSIONS

As of today, the problem of NSAID-induced lesions of the GIT remain a relevant issue. This is due to the fact that the pathogenic mechanisms of this process are still

unclear. All the GIT related risk factors should be taken into consideration by physicians of all specialties during their routine medical practice. The examination of the GIT should be performed regularly to prevent complications. Uncontrolled, long-lasting, unprescribed NSAID usage should draw the attention of doctors, especially in patients with comorbid states.

Diclofenac remains "Golden standard" of unselective NSAIDs and has shown the lowest rate of GIT side effects, when compared to ibuprofen and naproxen.

One of the most prescribed selective NSAID is nimesulide. It has a high safety profile, regarding GIT SEs on par with meloxicam and celecoxib, but has high hepatotoxicity.

The highest safety profile regarding GIT hemorrhage risks was seen in specific COX-2 inhibitors – coxibs. But their use was associated with a higher CV risk, so they should not be recommended to patients with cardiovascular diseases.

Taking all of this into consideration, timely prevention, and diagnosis of NSAID induced damage of the mucous membrane of the GIT, as well as rational and individually tailored NSAID prescription. The search for safer, less damaging to the GIT, with lower CV risk and a nephron- and hepatotoxicity is still ongoing. Until they are developed, if the use of NSAIDs is inevitable, management of patients with comorbid states should include additional measures to prevent complications.

REFERENCES

1. Parfitt J., Driman D. Pathological effects of drugs on the gastrointestinal tract: are view. HumPathol. 2007; 38(4):527–536. doi: 10.1016/j.humpath.2007.01.014.
2. Iee M., Feldman M. The aging stomach: implications for NSAID-gastropathy. Gut. 1997; 41(4):425–426. doi: 10.1136/gut.41.4.425.
3. Morozova T. E., Rykova S. M., Chukina M. A. NPVP-gastropatii u bol'nykh s sochetannoj patologiej serdechno-sosudistoj sistemy I zabolevaniyami sustavov I pozvonochnika. [NSAID gastropathy in patients with comorbid diseases].Experimental and clinical gastroenterology. 2015;118(6):64-70. (In Russian).
4. Novikov V. E., Kryukova N. O., Krikova A. V. NPVP indutsirovannyye gastropatii ikh profilaktika. [NSAID-induced gastropathies and their prevention].Reviews on clinical pharmacology and drug therapy. 2008;6(1):26-30. (In Russian).
5. Scarpellini E., Vos R., Blondeau K. et al. The effects of itopride on esophageal motility and lower esophageal sphincter function in man. Aliment. Pharmacol. Ther. 2011;33(1):99-105. doi: 10.1111/j.1365-2036.2010.04487.x.
6. Maev I.V., Andreev D.N., Dicheva D.T. Gastropatii, inducirovannye nesteroidnymi protivovospalitel'nymi sredstvami: patogeneticheski obuslovlennye podhody k profilaktike i terapii [NSAID-induced Gastropathies: Pathogenetically Substantiated Approaches to Prevention and Therapy]. Pharmateka. 2016;2:49–54. (In Russian).
7. Lanza F., Chan F., Quigley E. Guidelines for Prevention of NSAID-Related Ulcer Complications. Am J Gastroenterol. 2009; 104(3):728–738.
8. Lapina T.L. Gastropatiia, indutsirovannaia nesteroidnymi protivovospalitel'nymi preparatami: puti resheniia problemy. [Non-steroidal anti-inflammatory drug-induced gastropathy: solutions]. Rus. med. zhurn. 2009;2:54–7. (In Russian).
9. Chan F. The David Y. Graham lecture: use of non-steroidal anti-inflammatory drugs in a COX-2 restricted environment. Am J Gastroenterol. 2008;103(1):221–227. doi: 10.1111/j.1572-0241.2007.01545.x.
10. Karateev A.E., Nasonov E.L., Ivashkin V.T. et al. Racionalnoe-ispolzovanie nesteroidnykh protivovospalitelnykh preparatov. Klinicheskie rekomendacii. [Rational use of non-steroidal anti-inflammatory drugs. Clinical guidelines]. Rheumatology Science and Practice. 2018;56:1–29. (In Russian).
11. Lanas A., Garcia-Tell G., Armada B., Oteo-Alvaro A. Prescription patterns and appropriateness of NSAID therapy according to gastrointestinal risk and cardiovascular history in patients with diagnoses of osteoarthritis. BMC Med. 2011;9(1):38. doi: 10.1186/1741-7015-9-38.
12. Anders H., Vielhauer V. Renal co-morbidity in patients with rheumatic diseases. Arthritis ResTher. 2011;13(3):222–232.
13. Blackler R., Gemici B., Manko A., Wallace J. NSAID-gastroenteropathy: new aspects of pathogenesis and prevention. Curr Opin Pharmacol. 2014;19:11-6. doi:10.1016/j.coph.2014.05.008.
14. Kursov S.V., Nikonov V.V. Tsiklooksigenaza: fiziologicheskie effekty i, deystvie ingibitorov i perspektivy i dalneyshego ispolzovaniya paratsetamola (analiticheskiy obzor). [Cyclooxygenase:physilogical effects, inhibitors action and perspectives of paracetamol usage]. Medicina neotloznyh sostoanij. 2016;5(76):27-35. doi:10.22141/2224-0586.5.76.2016.76430. (In Russian).
15. Lazebnik L.B., Belova G.V. Sistematiziruyuschaya klassifikatsiya multifokalnyih povrezhdeniy slizistoy obolochki pischevaritelnogo trakta nesteroidnyimi protivovospalitelnyimi NPVP I antitromboticheskimi preparatami «Moskovskaya klassifikatsiya». [Systematizing classification of multifocalle sions of the mucosa of the digestive tract by non-steroidal anti-inflammatory and anti-thrombotic drugs]. Eksperimental'naiai klinicheskaia gastroenterologiia. 2018;(151):19-27. (In Russian).
16. Barinov E., Sulaieva O., Lyakch Y. et al. Platelet aggregation measurement for assessment of hemostasis failure mechanisms in patients with gastroduodenal ulcer bleeding. Clin Exp Gastroenterol. 2013;5(6):139-48. doi:10.2147/CEG.S45685.
17. Balukova E.V. NPVP-indutsirovannoy gastropatii: ot ponimaniya mehanizmov razvitiya k razrabotke strategii profilaktiki I lecheniya. [NSAID-induced gastropathy: from understanding of development mechanisms to developing a prevention and treatment strategy]. RMJ. 2017;25(10):697-702. (In Russian).
18. Jarosz M., Szkaradek N., Marona H. et al. Evaluation of anti-inflammatory and ulcerogenic potential of zinc-ibuprofen and zinc-naproxen complexes in rats. Inflammopharmacology. 2017;25(6):653-663. doi:10.1007/s10787-017-0361-0.
19. Lim Y., Lee J., Ku Y. et al. Rescu strategies against non-steroidal anti-inflammatory drug-induced gastroduodenal damage. J Gastroenterol Hepatol. 2009;24(7):1169-78. doi:10.1111/j.1440-1746.2009.05929.x.
20. Musumba C., Pritchard D.M., Pirmohamed M. Reviewarticle: cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment Pharmacol Ther. 2009; 15;30(6):517-31. doi:10.1111/j.1365-2036.2009.04086.x.
21. Wallace J. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? Physiol Rev. 2008;88(4):1547-65. doi:10.1152/physrev.00004.2008.
22. Balabantseva A.P., Kliaritskaia I.L., Tsapyak T.A. et al. Sovremennyiy podhod k pervichnoy profilaktike NPVP-gastropatiy i enteropatiy. [Modern approaches to primary preventionof NSAID-gastropathy and enteropathy]. Crimean Journal of Internal Disease. 2015;(26):31-38. (In Russian).

23. Avramenko A.A., Gozhenko A.I., Goidyk V.S. Iazvennaiabolezn': ocherkiklinicheskoipatofiziologii [Peptic ulcer disease:essays on clinical pathophysiology]. Odessa; 2008, p.304. (In Russian).
24. Tsimmernan Ia.S. Gastroenterologiia: rukovodstvo [Gastroenterology: a guide]. Moscow: GEOTAR-Media; 2012, p. 800. (in Russian).
25. Evseev M.A. Protivovospalitelnoe medikamentoznoe povrezhdenie kishechnoy trubki: klinicheskoe znachenie, patogenez, vozmozhnosti profilaktik.[Anti-inflammatory drug-induced injury to the intestinal tube: clinical value, pathogenesis, possibilities of prevention]. Nevrologiya, Neiropsikhiatriya, Psikhosomatika. 2013;(1):79-87. (In Russian).
26. Bjarnason I., Takeuchi K. Intestinal permeability in the pathogenesis of NSAID-induced enteropathy. J Gastroenterol. 2009;44(19):23-29. doi:10.1007/s00535-008-2266-6.
27. Podpletniaia E.A., Mamchur V.I. Mechanisms of gastroduodenotoxicity of non-steroidal anti-inflammatory drugs. Journal of the National Academy of Medical Sciences of Ukraine. 2005;(1):47-62. (In Russian).
28. Uspenskiy Y.P., Baryshnikova N.V., Orlov O. Y. et al. NPVP-assotsiirovannaya patologiya zheludochno-kishechnogo trakta: vyibor preparata, taktika vedeniya patsientov. [NSAID-associatedpathologyofthegastrointestinaltract: choiceofthedrug, tacticsofpatientmanagement]. Spravochnikpoliklinicheskogovracha. 2014;(8):42-47. (In Russian).
29. Lanas Á., Carrera-Lasfuentes P., Arguedas Y. et al. Risk of upper and lower gastrointestinal bleeding in patients taking non-steroidal anti-inflammatory drugs, antiplatelet agents, or anticoagulants. Clin Gastroenterol Hepatol. 2015;13(5):906-12.e2. doi:10.1016/j.cgh.2014.11.007.
30. Sulaieva O.N., Wallace J. L. Novaya strategiya gastrointestinalnoy protektsii s ispolzovaniem gazoobraznyih mediatorov. [New strategy for gastrointestinal protection based on gaseous mediators application]. Russian Journal of Gastroenterology, Hepatology, Coloproctology. 2016;26(3):17-23. (In Russian).
31. Klyaritskaya I.L., Balabantseva A.P., Fursova V.A. Enteropatii, indutsirovannyie priemom nesteroidnyih protivovospalitelnyih preparatov. [Enteropathies induced by intake of non-steroidal anti-inflammatorydrugs]. Crimean Journal of Internal Disease. 2014;(22):38-44. (In Russian).
32. Gubska O.Ju., Kuzminets A.A. NPZP-enterotoksichnist: fokusna problem. [NSAID enterotoxicity: focus on the problem]. Modern gastroenterology. 2018;(103):50-59. doi:10.30978/MG-2018-5-50. (in Ukrainian).
33. Watanabe T., Higuchi K., Kobata A. et al. Non-steroidalanti-inflammatory drug-induced small intestinal damageis Toll-likereceptor 4 dependent. Gut. 2008;57(2):181-7. doi:10.1136/gut.2007.125963.
34. Tkach S., Onischuk L., Balabantseva A. Efficacy and safety of Rebamipide in prevention of NSAID-gastropathy. International Journal of Biomedicine. 2017;7(1):57-59. doi:10.21103/article7(1)_shc1.
35. Collaborative overview of randomised of antiplateled therapy - -I: Prevention of death, myocardial infarction and stroke by prolonged antiplateled therapy in various categories of patients. Antiplatelet Trialists`Collaboration. BMJ. 2014;308(6921):81–106. PMCID: PMC2539220
36. Lanas A., Perez-Aisa M., Feu F. et al. A nationwide study of mortality associated with hospital admission due to severe gastrointestinal events and those associated with non steroidalantiinflammatorydrug use. Am. J. Gastroenterol. 2015; 100(8): 1685–93. doi: 10.1111/j.1572-0241.2005.41833.x.
37. Srinivasan A., DeCruz P. A practical approach to the clinical management of NSAID enteropathy. Scand. J. Gastroenterol. 2017;52(9):941—947. doi: 10.1080/00365521.2017.1335769.
38. Bjarnason I. et al. Effect of non-steroidal anti-inflammatory drugs on the human small intestine. Drugs.1986; 32 (1):35-41.
39. Bjarnason I., Hayllar J. Side effects of non-steroidal anti-inflammatory drugs on the small and large intestinein humans.Gastroenterology. 1993;104(6):1832-1847. doi: 10.1016/0016-5085(93)90667-2.
40. Yusuf S., Zhao F., Mehta S. et al. Effect of clopidogrel in addition to aspirinin patients with acute coronary syndromes without ST elevation. The CURE trial investigators. N Engl J Med 2001; 345(7): 494-502.
41. Korneeva O.N., Drapkina O.M. Profilaktika zheludochno kishechnyh oslozhnenij pri prieme anti trombocitarnyh preparatov. [The prevention of gastrointestinal complications in patients taking antiplatelet drugs]. Dokazatel'naya gastroehnterologiya, 2012; 2: 77–81. (In Russian).
42. Kubler W., Darius H. Primary prevention of coronary heart disease with aspirin. J. Kardiol. 2015; 94(3): 66–73. doi: 10.1007/s00392-005- 1309-8.
43. Henry D., Dobson A., Turner C. Variability in the risk of major gastrointestinal complications from non aspirin non-steroidal anti-inflammatory drugs. Gastroenerology. 2010; 105(4): 1078–88.
44. ACCF/ACG/AHA 2008 Expert Consensus Document on Reducing the Gastrointestinal Risks of Antiplatelet Therapy and NSAID Use. J Am Coll Cardiol 2008;52(18):1502–17. doi: 10.1016/j.jacc.2008.08.002.
45. Patrono C., Bachmann F., Baigent C. et al. Expert consensus document on the use of antiplatelet agents. The task force on the use of antiplatelet agents in patients with atherosclerotic cardiovascular disease of the European society of cardiology. Eur Heart J. 2004;25(2):166-81.
46. Patrono C., Baigent C., Hirsh J., Roth G. American College of Chest Physicians. Antiplatelet Drugs. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition).Chest 2008; 133(6):199–233.doi: 10.1378/chest.08-0672.
47. Garcia Rodrigues L. Variability in risk of gastrointestinal complications with different non-steroidal anti-inflammatory drugs. Am. J. Med. 1998;104(3):30–34.
48. Singh G., Rosen Ramey D. NSAID induced gastrointestinal complications: the ARAMIS perspective. J. Rheumatology. 1998:25(51):8–16.
49. Trelle S., Reichenbach S., Wandel S. et al. Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis. BMJ. 2011; 342:1-86.
50. Mattia C., Coluzzi F. COX-2 inhibitors: pharmacological data and adverse effects. Minerva Anesthesiology. 2005; 71(7–8):461–470.
51. Hawkey C., Kahan A., Steinbrück K. et al. Gastrointestinal tolerability of meloxicam compared to diclofenac in osteoarthritis patients. International MELISSA Study Group. Meloxicam Large-scale International Study Safety Assessment. British J. Rheumatol. 1998;37(9): 937–945.
52. Lazebnik L.B., Golovanova E.V., Alekseenko S.A. et al. Rekomendatsii po profilaktike i lecheniyu ezofago-gastro-entero-kolopatiy, indutsirovannyih nesteroidnyimi protivovospalitelnyimi preparatami (NPVP). [Recommendations for the prevention and treatment of esophagogastro-entero-colopathy induced by non-steroidal anti-inflammatory drugs "NSAID"]. Experimental and Clinical Gastroenterology. 2018; 151(3): 4–18. (in Russian).
53. Bunczak-Reeh M., Hargreaves K. Effect of inflammation on the delivery of drugs to dental pulp. J. Endod. 1998;24(12):822–824.
54. Schmidt M., Lamberts M., Olsen A. et al. Cardiovascular safety of non-aspirin non-steroidal anti-inflammatory drugs: review and position paper by the working group for Cardiovascular Pharmacotherapy of the European Society of Cardiology. Eur. Heart J. Cardiovasc. Pharmacother. 2016;2(2):108–118.
55. Conforti A., Leone R., Moretti U. et al. Adverse drug reactions related to the use of NSAIDs with a focus on nimesulide: results of spontaneous reporting from a Northern Italian area. Drug Saf. 2001; 24(14):1081–1090.

56. Laporte J., Ibanez L., Vidal X. et al. Upper gastrointestinal bleeding associated with the use of NSAIDs: new erversus old eragents. Drug Safety. 2004;27(6):411–20. doi: 10.2165/00002018-200427060-00005.
57. Boelsterli U. Mechanisms of NSAID-induced hepatotoxicity: focus on nimesulid. Drug Saf. 2002;25(9):633–648.
58. Andrade R., Lucena M., Fernandez M. et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology. 2005;129(2):512–521.
59. Traversa G., Bianchi C., Da Cas R. et al. Cohort study of hepatotoxicity associated with nimesulide and other non-steroidal anti-inflammatory drugs. BMJ. 2003; 327(7405):18–22. doi: 10.1136/bmj.327.7405.18.
60. Rostom A., Goldkind L., Laine L. Nonsteroidal anti-inflammatory drugs and hepatictoxicity: a systematic review of randomized controlled trial sin arthritis patients. Clin Gastroenterol Hepatol. 2005;3(5):489-98. doi:10.1016/s1542-3565(04)00777-3.
61. Assessment report for Nimesulide containing medicinal products for systemic use (2012) 20 January 2012, EMA/73856/2012, Procedure number: EMEA/H/A-31/1261 (http://www.ema.europa.eu/).
62. Venegoni M. et al. Effects of the European restrictive actions concerning nimesulide prescription: a simulation study on hepatopathies and gastrointestinal bleedings in Italy. Ann Ist Super Sanita. 2010;46(2): 153–157.
63. Kwon J., Kim S., Yoo H., Euni L. Nimesulide-induced hepatotoxicity: A systematic review and meta-analysis. PLoS One. 2019;14(1):e0209264. doi:10.1371/journal.pone.06209264.
64. Cannon C., Curtis S., FitzGerald G. et al. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Longterm (MEDAL) programme: a randomised comparison. Lancet. 2006;368(9549): 1771-81.
65. Laine L., Curtis S.P., Cryer B. et al. Assessment of upper gastrointestinal safety of etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison. Lancet. 2007; 369(9560):465-73.
66. Laine L., Curtis S., Langman M. et al. Lower gastrointestinal events in a doubleblind trial of the cyclo-oxygenase-2 selective inhibitor etoricoxib and the traditional non steroidal anti-inflammatory drug diclofenac. Gastroenterology. 2008; 135(5):1517-25. doi: 10.1053/j.gastro.2008.07.067.

ORCID and contributionship:
Yana Y. Hnepa: 0000-0002-0276-1466 [A,B,D]
Ivan V. Chopey: 0000-0003-4626-0855 [B,E,F]
Ksenia I. Chubirko: 0000-0002-4379-0538 [E,F]
Andriy M. Bratasyuk: 0000-0003-4390-2357 [E]

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CORRESPONDING AUTHOR
Yana Y. Hnepa
Uzhhgorod National University
148 Sobranetska st., 88017 Uzhhgorod, Ukraine
tel: +380935488978
e-mail: yana.hnepochka@gmail.com

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REVIEW ARTICLE

DYNAMICS OF DIPHTHERIA INCIDENCE IN THE TRANSCARPATHIAN REGION OF UKRAINE IN THE VACCINATION ERA

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Yelyzaveta I. Rubtsova[1], Svitlana V. Oberemko[2], Maryana M. Rosul[1]
[1]UZHGOROD NATIONAL UNIVERSITY, FACULTY OF POSTGRADUATE AND PRE-UNIVERSITY EDUCATION, UZHGOROD, UKRAINE
[2]TRANSCARPATHIAN REGIONAL LABORATORY CENTER OF THE MINISTRY OF HEALTH OF UKRAINE, UZHGOROD, UKRAINE

ABSTRACT

The aim: Was to study the dynamics of the incidence of diphtheria in the Transcarpathian region of Ukraine.

Materials and methods: A retrospective analysis of the data of the state statistical reporting of the Transcarpathian region on the incidence of diphtheria, the carriage of infection, vaccination coverage and the severity of diphtheria immunity was carried out.

Conclusions: Low coverage of the population with vaccination against diphtheria, registration of an outbreak of diphtheria among international students of the region, and other regions of Ukraine in conditions of increased population migration, are alarming prognostic signs of the possible development of another significant epidemic rise of diphtheria morbidity in Transcarpathia.

KEY WORDS: diphtheria, morbidity, carrier, epidemic, vaccination

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INTRODUCTION

Diphtheria is a dangerous infectious disease with a high mortality and disability rate. The source of infection is a sick person or a bacteria carrier of toxigenic strains, sick children and adults who do not have antitoxic immunity, including those vaccinated with low antibody titers. from The case-fatality rate from diphtheria is 5-17%, even in individuals receiving proper care and treatment. [1] This figure can reach 20% in children under five years of age and adults over 40 years. [2]

Mass immunization of the population against diphtheria, which has been carried out since the mid-50s of the last century, has contributed to a sharp decline in the pathogen's incidence and bacteria carriers. However, diphtheria outbreaks, although rare, still occur worldwide in the last decade as in India, Nigeria, in refugee resettlement centers in Bangladesh, in Yemen and other Middle East countries. [3,4,5,6,7]

Diphtheria remains endemic in many areas with limited vaccination coverage. In 2018, the WHO recorded 16,611 reported cases. Generally, diphtheria is under-reported from many regions, including Asian, African and Eastern Mediterranean countries. [8]

The epidemiology of diphtheria is constantly changing owing to varying prevalence of Corynebacterium spp. in different regions, travel and migration, variations in socioeconomic status and vaccination rates and other factors. Humans are the only known natural host for C. diphtheria. [2,8] Though the toxigenic strain Corynebacterium ulcerans has been increasingly identified as an etiological agent in Western Europe, whereas disease associated with it is of zoonotic origin (from domestic and wild mammals). High-resolution molecular typing of strains is vital to identify disease transmissions and outbreaks. [2,9,10]

At the end of the last century, there were two diphtheria waves in Europe: in 1982–1985 and 1990–1999. [1] The largest outbreak in recent years occurred between 1990 and 1998 in the states of the former Soviet Union. More than 158,000 cases of diphtheria were reported in post-Soviet countries between 1990 and 1999, of which 4,000 were fatal [11,12]

In Ukraine, there were more than 20,000 diseases, 700 deaths and 13,204 cases of carriers of toxigenic strains of the pathogen detected during this period. The emergence of the epidemic was facilitated by the low level of coverage of children and adults with preventive vaccinations. The diphtheria vaccination rate declined significantly in the early 1990s due to political unrest after the collapse of the Soviet Union. This has led to an explosive increase in morbidity and mortality from this infection. Lack of caution about diphtheria (many primary care physicians have not encountered it before) and lack of physicians' awareness have led to delays in diagnosing and treating patients. [11]

Simultaneously, all types of migration processes in Ukraine have significantly intensified in recent decades, according to the National Report of the Institute of Demography and Social Research of the Academy of Sciences of Ukraine. [13] This process is highly typical for Transcarpathial region of Ukraine too. [14]

The epidemic process of diphtheria in mass vaccination conditions is manifested by sporadic cases of diseases and

registration of bacteria carriers. Laboratory bacteriological diagnosis is a leading factor in the epidemiological surveillance of diphtheria. Undetected and untreated carriers of the pathogen create conditions for the spread of infection. The high proportion of vaccinated does not prevent colonisation by the pathogen of the nasopharyngeal mucosa, so it is important to conduct screening studies to maximise the detection and remediation of carriers of C. diphtheria. The majority of carriers are carriers of nontoxigenic strains, some of which have the toxigenic gene. [2,8]

The frequency of detection of bacteria carriers in the post-epidemic period decreases every year. A serious harbinger of the diphtheria epidemic of the 1990s was the increase in the registration of carriers of toxigenic strains of the pathogen. The ratio of patients with diphtheria to carriers 1:2 and more indicates latent morbidity due to increased carriers and errors in diagnosis. [15]

The study of the epidemiological significance of the detected carrier of diphtheria in Kiyiv in 1989-1998 showed that the levels of carriers of toxigenic and nontoxigenic strains of C. diphtheria differed significantly among different groups of the population. The highest group of epidemic risk were patients with pathology of the nose and throat. [15] Before the beginning of the epidemic in 1991, there was a spread of nontoxigenic strains of C. diphtheria. The beginning of the epidemic coincided with the beginning of an increase in the intensity of circulation of toxigenic strains of the pathogen in the population. [15,16]

Selective genetic studies of non-toxigenic strains of the diphtheria pathogen found that 7-10.5% of them had a toxigenicity gene and were able to cause disease. And in 1999 in Ukraine in 17.3% of patients with diphtheria were isolated nontoxigenic strains of the pathogen. The largest number of such strains was registered in patients diagnosed with diphtheria in Kyiv, where out of 90 initially examined patients, in 43 nontoxic strains were identified (47.8%). [11,13,14]

Therefore, all such phenotypically nontoxigenic strains were recommended for study by the polymerase chain reaction method for in-depth investigation and identification of the possible toxigenicity gene. [2,8]

According to the Ukrainian Center of the State Sanitary and Epidemiological Surveillance, over the last 20 years, there has been a decrease in diphtheria registration, even against the background of a significant decrease in the volume of preventive vaccinations. In some cases, this was probably due to the "cure" of diphtheria with modern antibiotics in the early catarrhal period of the disease and the removal of the previous diagnosis of "diphtheria" in the absence of a positive microbiological study. There were cases when in patients with a negative bacteriological examination the diagnosis of diphtheria was removed and clarified for the diagnosis of "Follicular tonsillitis" in the existing clinical picture and specific treatment of the patient (introduction of diphtheria serum), which led to artificial reduction of morbidity [15].

In the system of epidemiological surveillance of diphtheria, immunoprophylaxis is the most effective measure. According to the vaccination calendar, coverage of the population with preventive vaccinations has decreased from year to year for the last three decades. This process was due to untimely and irregular delivery of immunobiological drugs. [11,17,18] Anti-vaccine propaganda and refusal to vaccinate, untimely vaccination, violation of vaccination deadlines also contributed to that. [18] As a result, the number of immunologically vulnerable to diphtheria population and likelihood of epidemic outbreaks is increased in the country. These processes prompted us to study this issue in our region.

THE AIM

The aim was to investigate the peculiarities of the diphtheria epidemic process in the Transcarpathian region of Ukraine in the era of vaccination.

MATERIALS AND METHODS

The study material was indicators of diphtheria incidence, carriers of the bacteria, diphtheria vaccination coverage and the results of studies of diphtheria herd immunity in the Transcarpathian region from the following sources of information:

1. Official site of the main department of statistics in the Transcarpathian region. http://database.ukrcensus.gov.ua
2. State statistical reporting form №2 "Report on individual infections and parasitic diseases" in the Transcarpathian region for 2000-2020.
3. Sectoral reporting f.№ 40 of the Transcarpathian Regional Sanitary and Epidemiological Service and the Laboratory Center of the Ministry of Health of Ukraine for 2010-2020.
4. State statistical reporting on preventive vaccinations (Form 70) for 2010-2020.
5. Computerized reporting program on preventive vaccinations "UKRVAK" in the Transcarpathian region for 2010-2020.

The study data were processed using a comprehensive epidemiological method (CEM), including descriptive-evaluative, analytical methods and forecasting. A retrospective epidemiological analysis was performed. Data from a complex of bacteriological and serological tests were used.

REVIEW AND DISCUSSION

Since the introduction of state registration of infectious diseases in the Transcarpathian region (1946), diphtheria's dynamics was wavy. For the period 1946-1959, the figures ranged from 41.2 per 100.000 population (328 cases) in 1948 to 13.7 (120 cases) in 1955. During the 1960s and 1980s, against the background of mass coverage of the pediatric population with diphtheria vaccination, the incidence was sporadic, but cases were registered annually. The highest incidence of diphtheria in this period was observed in 1986 and 1987, 0.08 and 1.2 per 100 thousand people, respectively. (see Figure 1)

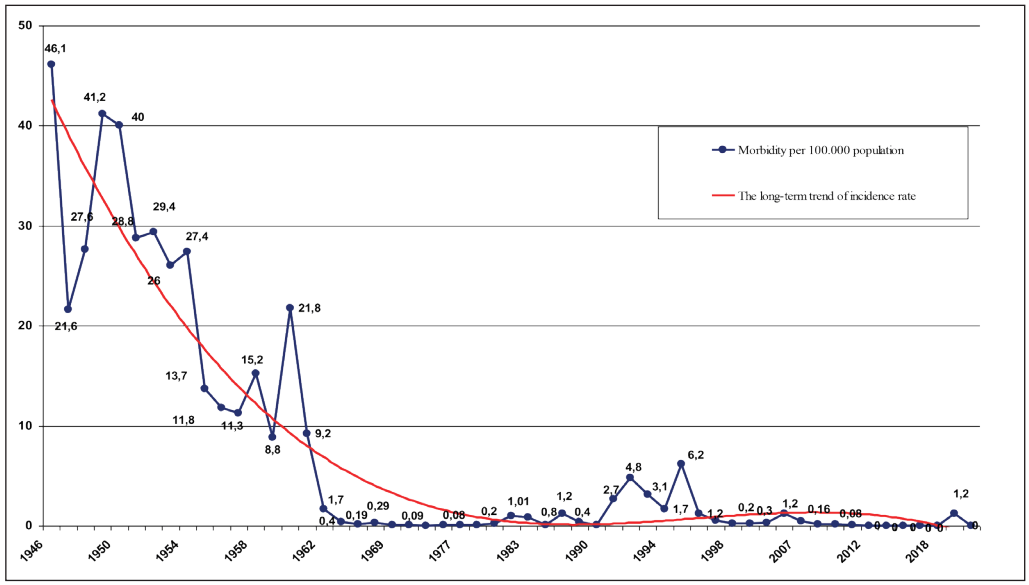

Fig. 1. Dynamics of the incidence of diphtheria (per 100.000 population) and its tendency in the Transcarpathian region for the period 1946-2020

Within the epidemic rise in diphtheria in the 90s of the last century in Transcarpathia, 266 cases of the disease were registered, including 96 in children under 14 years (42.5%), and 7 deaths (3.1%). During this period, 956 carriers of toxigenic strains of diphtheria were detected, including 322 in children (33.7%). The highest rates of disease at this time were observed in 1992 (61 cases, 4.8 per 100.000 population) and 1995 (79 cases, 6.2 per 100.000 population, the peak of the last epidemic).

In 2000-2004, no diphtheria disease was detected. Then 25 cases of infection were diagnosed within 2005-2011. Intensive incidence rates ranged from 0.16 to 1.2 per 100.000 people.

Simultaneously, no diphtheria carrier was found among 51,454 people examined for tonsillitis and other otorhinolaryngological pathology. Healthy individuals were also examined for prophylactic purposes before admission to closed organized groups.

In 2019, a diphtheria outbreak was registered again – 15 cases.

This happened in the fall of 2019 in Uzhgorod among foreign students from India. 15 people fell ill. The diagnosis of patients was: Diphtheria of the tonsils, localized, islet form, gram + strain Corynebacterium Mitis, toks-. One patient had a course of moderate severity; others had a mild course of the disease.

According to the results of molecular genetic studies of selected cultures of Corynebacterium diphtheriae specific fragments of nucleic acids of the diphtheria toxin gene were found in three patients.

Of the 15 patients, 14 were identified actively during a set of anti-epidemic measures for the first patient. The source of infection of the first patient is unknown. However, shortly before the disease, he was temporarily in other countries (Germany, Israel, India). All patients had no documented evidence of diphtheria vaccination.

106 contacts were surveyed and no carrier was invented. No patients or carriers were found among the local population.

In total, 16,007 people, including 2,848 university students, were vaccinated against diphtheria during the period of localization and elimination of the outbreak in the city of Uzhgorod.

The dynamics of diphtheria carriers' detection for the period 1990-1999 in the Transcarpathian region was characterized by the highest level in 1993-1995 (11.1-12.5 per 100.000 population) The average annual level of carriers for this period was 3.67 per 100 thousand people. The next decade (2000-2010) decreased 3.7 times to 0.98 per 100 thousand people. 3 carriers of nontoxigenic strains of the diphtheria pathogen were identified in 2010-2020.

During 2010-2020, bacteriological laboratories of the Sanitary and Epidemiological Service and medical institutions in Transcarpathian region examined 75068 (18+, 0,02%) people, of whom 69,9% for diagnostic purposes, 4,9% – for epidemic indications and 25,2% – for prophylactic purposes. On average, 7.5 thousand people are examined annually.

Coverage of the region's population by planned preventive vaccinations against diphtheria for 2010-2018 was deficient. The most critical situation was in 2015; then vaccination plan was implemented by only 7.2%. It's necessary to note that this was mainly due to the adult population's low vaccination coverage with fluctuations from 0 in 2015 to 68.9% in 2018. Since 2018, the situation with the implementation of vaccinations has improved significantly. However, the powerful layer of immunologically diphtheria unprotected population of our region formed in recent years remained a serious factor in the possible development and spread of the diphtheria epidemic. (see Table I).

Due to the diphtheria outbreak in 2019, the implementation of diphtheria vaccination plans has increased significantly, including a significant percentage of diphtheria vaccination coverage in adults (96.4%). In 2020, there is a decrease in planned vaccination volume owing to the spread of the COVID-19 pandemic.

The study of the state of population immunity of the Transcarpathian region has been conducted from 1987 to

Table I. Implementation of the consolidated plan of diphtheria vaccinations in the Transcarpathian region of Ukraine within 2010-2020

Years	Plan	Executed		Not fulfilled	
		n	%	n	%
2010	121527	61344	50,5	60183	49,5
Adult population	52135	14340	27,5	37795	72,5
2011	167596	39151	23,4	128445	76,6
Adult population	83811	7800	9,3	76011	90,7
2012	228083	105719	46,3	122364	53,6
Adult population	112581	41620	36,9	70961	63,0
2013	127721	42657	33,4	85064	66,5
Adult population	48168	7726	16,04	40442	83,9
2014	132976	13060	9,8	119916	90,2
Adult population	47352	854	1,8	46498	98,2
2015	169351	12144	7,2	157207	92,8
Adult population	36499	0	0	36499	100,0
2016	218487	34030	15,7	184457	84,3
Adult population	53210	1963	3,7	51247	96,3
2017	252551	95093	37,7	157458	62,3
Adult population	103294	39665	38,5	63629	61,5
2018	160998	93675	58,1	67323	41,9
Adult population	49736	34284	68,9	15452	31,2
2019	215560	174391	80,9	41169	19,1
Adult population	89327	86096	96,4	3231	3,6
2020	198974	77514	38,9	121460	61,1
Adult population	87861	27339	31,1	60552	68,9

2003 by assessment of the titers of diphtheria antibodies in blood serum. Unfortunately, further studies were done in insufficient volumes for statistically significant conclusions. We reveled two waves of rising levels of vulnerability to this infection within this period. In 1990 23.9% of the population had immune defense against diphtheria and 25.7% in 1994. Comparison of the state of immunity with diphtheria incidence dynamics revealed a pattern: the years with the highest percentages of immunologically vulnerable population preceded the increase in diphtheria incidence: in 1991 – 35 cases; in 1992 – 61; in 1993 – 40 cases. The second peak of population insecurity preceded by an outbreak of diphtheria in 1995 – 79 cases.

With the introduction of preventive vaccinations against diphtheria in the Transcarpathian region in the 50-60 years of the last century, the infection gradually ceased to be childhood. Analysis of the age structure of the prevalence, carriage and morbidity of diphtheria during the epidemic of the '90s shows slight differences in the incidence of children and adults: the proportion of children was 52.2%, adults – 47.8%. Regarding the carrier of diphtheria pathogens, 58.3% of the detected carriers were children, and 41.7% were adults. The characteristics of the age distribution of patients during the epidemic of the '90s were as follows: adult population 67.5%, children under 14 years – 32.5%.

According to the retrospective analysis results, despite many years of mass vaccine prophylaxis, diphtheria has retained the periodicity and seasonality characteristic of the pre-vaccination era. Analysis of seasonality of incidence diphtheria for the epidemic period of 1990-2000 in the Transcarpathian region shows an uneven annual distribution of morbidity and the manifestation of the characteristic autumn-winter seasonality: 64% of registered cases were registered in the period from November to April.

CONCLUSIONS

At the present stage, diphtheria's epidemic process in the Transcarpathian region is characterized by the following features. Namely, a long period with a minimal level of morbidity and its absence during 2012-2018 (the last case was detected in 2011), followed by an epidemic outbreak in 2019. The Surveillance Service registered the minimum level of carriers of mainly nontoxigenic diphtheria strains in the absence of registration of the disease. A significant reduction in the volume of preventive vaccinations against diphtheria is characteristic for last decade in all age groups. Low coverage of the population with vaccination against diphtheria, registration of an outbreak of diphtheria among international students of the region, and in other regions of Ukraine in conditions of increased population migration,

are alarming prognostic signs of the possible development of another significant epidemic rise of diphtheria morbidity in Transcarpathia.

The results of the analysis of the diphtheria epidemic leading risk factors indicate the need for timely, comprehensive implementation of measures to prevent and combat this infection.

REFERENCES

1. Wagner K.S., White J.M., Lucenko I. et al. Diphtheria in the postepidemic period, Europe, 2000–2009. Emerg. Infect. Dis. 2012;18(2):217–225.
2. Sharma N.C., Efstratiou A., Mokrousov I. et al. Diphtheria. Nat Rev Dis Primers. 2019; 5:81. https://doi.org/10.1038/s41572-019-0131-y.
3. Murhekar M. Epidemiology of diphtheria in India, 1996–2016: implications for prevention and control. Am J Tropical Med Hyg. 2017;97(2):313–318. doi: 10.4269/ajtmh.17-0047.
4. Besa N. C., Coldiron M. E., Bakri A. et al. Diphtheria outbreak with high mortality in northeastern Nigeria. Epidemiol. Infect. 2013;142(4):797–802. doi: 10.1017/S0950268813001696.
5. Rahman M. R., Islam K. Massive diphtheria outbreak among Rohingya refugees: lessons learnt. J Travel Med. 2019;26(1). doi: 10.1093/jtm/tay122.
6. Dureab F., Al-Sakkaf M., Ismail O. et al. Diphtheria outbreak in Yemen: the impact of conflict on a fragile health system. Confl Health. 2019;13:19. doi: 10.1186/s13031-019-0204-2.
7. Raad I. I., Chaftari A.-M., Dib R.W. et al. Emerging outbreaks associated with conflict and failing healthcare systems in the Middle East. Infect Control Hosp Epidemiol. 2018;39(10):1230-1236. doi: 10.1017/ice.2018.177.
8. World Health Organization. Diphtheria vaccine: WHO position paper — August 2017. Wkly Epidemiol Rec. 2017;92(31):417-35.
9. Rengganis I. Adult diphtheria vaccination. Acta Med Indonesia. 2018;50(3):268–272.
10. van Dam A. P., Schippers E.F., Visser L.G. et al. A case of diphtheria in the Netherlands due to an infection with Corynebacterium ulcerans. Ned Tijdschr Geneeskd. 2003;147(9):403-406.
11. Kolesnikova I.P., Mokhort H.A., Kolesnikov M.M. et al. Zahalna otsinka stanu zakhvoriuvanosti na vaktsyno kerovani infektsii v Ukraini (1944-2014 rr.), za osnovnymy epidemiolohichnymy kryteriiamy. Medychna nauka Ukrainy. 2016;12(1-2):64-71. (In Ukrainian).
12. WHO Vaccine-Preventable Diseases: Monitoring System. 2008 Global Summary. Immunization, Vaccines and Biologicals. World Health Organization. Geneva, Switzerland; 2008, 385 p.
13. Ukrayinske suspilstvo: migracijnij vimir. Nacionalna dopovid /Institut demografiyi ta socialnih doslidzhen im. M. V. Ptuhi NAN Ukrayini. Kiyiv; 2018, 396 p. (In Ukrainian).
14. Bachinska M. V., Baranyak I. Ye. Suchasni tendenciyi migracijnoyi aktivnosti naselennya karpatskogo regionu .Ekonomika ta derzhava. 2019;12:71-76. (In Ukrainian).
15. Kozlova I.A., Mokhort H.A., Nosenko H.A. et al. Epidemiolohichne znachennia vyiavlennia korynebakterii dyfterii v m. Kyievi v period aktyvizatsii epidemichnoho protsesu (1989-1998rr.). Suchasni infektsii. 2000;4:25-30. (In Ukrainian).
16. Mokhort H.A. Proiavy epidemichnoho protsesu dyfterii v suchasnykh umovakh i shliakhy udoskonalennia epidemiolohichnoho nahliadu. AMN Ukrayini. In-t epidemiologiyi ta infekc. hvorob im. L.V.Gromashevskogo. Avtoreferat dys. k.m.n. Kiyiv; 2003, 20 p. (In Ukrainian).
17. Podavalenko A.P. Estimating the Complication Risk of Epidemic Situation with Diphtheria in Ukraine. Asian Journal of Epidemiology. 2018;11(1):26-33.
18. Zaslavska G.O., Dmitruk V.I. Dosyagnennya ta problemi imunoprofilaktiki infekcijnih zahvoryuvan u ditej na suchasnomu etapi. Pereshkodi v provedenni imunizaciyi naselennya. Aktualna infektologiya. 2017;5(5):200-204. (In Ukrainian).

ORCID and contributionship:

Yelyzaveta I. Rubtsova: 0000-0001-9395-1822 [A,B,D]
Svitlana V. Oberemko: 0000-0003-1107-7938 [B,C,F]
Maryana M. Rosul: 0000-0002-2106-5386 [C,F]

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CORRESPONDING AUTHOR

Yelyzaveta I. Rubtsova
Uzhgorod National University
14 Universitetskaya St., 88000 Uzhgorod, Ukraine
tel:+380509277849
e-mail:eli.rubtsova@cmail.com

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CASE STUDY

MICROSCOPIC POLYANGIITIS – A VIEW OF THE PROBLEM THROUGH THE LENS OF A NEPHROLOGIST

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Ivan P. Katerenchuk, Lydia A. Tkachenko, Tatiana I. Yarmola, Victoria V. Talash
UKRAINIAN MEDICAL STOMATOLOGICAL ACADEMY, POLTAVA, UKRAINE

ABSTRACT

The article presents two clinical cases of microscopic polyangiitis in patients with symptoms of glomerulonephritis with renal failure, which were preceded by such nonspecific symptoms as: abdominal syndrome, high blood pressure, arthralgia, myalgia, weight loss, uveitis, shortness of breath, general weakness. Clinical and laboratory-instrumental aspects of diseases are analyzed. Emphasized the feasibility of early diagnosis, adequate therapy appointment.

The aim of the article is to show that only with timely prescribing of pathogenetic therapy it is possible to achieve clinical and laboratory remission and, even, to cease hemodialysis sessions.

It was described two clinical cases of microscopic polyangiitis in patients with symptoms of glomerulonephritis with renal failure. Approaches to complex treatment of patients with the use of pathogenetic and the possibility of using renal replacement therapy were discussed. After verifying the diagnosis, all patients started immunosuppression with corticosteroids and cytostatics. It is shown that only with timely prescribing of pathogenetic therapy it is possible to achieve clinical and laboratory remission.

Clinical examples demonstrate to physicians that systemic vasculitis can often hide under the «mask» of other diseases and require timely diagnosis and immediate pathogenetic treatment.

KEY WORDS: microscopic polyangiitis, systemic vasculitis, glomerulonephritis, renal replacement therapy

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INTRODUCTION

Systemic vasculitis (SV) is a heterogeneous group of diseases where ischemia and tissue necrosis occur as a result of inflammation of the blood vessels (primary or secondary to the underlying disease), in the pathogenesis of which involves mechanisms of both immune and non-immune genesis. Among this group of diseases are vasculitis with a predominant lesion of small and medium vessels, which include anti-neutrophil cytoplasmic antibody (ANCA) -associated. They are united by a variety of multifaceted lesions, rapid progression and, often, adverse effects. [1, 2, 3].

ANCA-associated «renal» vasculitis is a common problem in nephrology [4, 5, 6].

ANCA is a heterogeneous population of autoantibodies that react with various neutrophil cytoplasmic enzymes with proteinase-3 (PR3) and myeloperoxidase (MPO), rarely with lactoferrin, cathepsin I and others. The indirect immunofluorescence reaction reveals: 1) cytoplasmic ANCA (cANCA), which are more common in granulomatosis with polyangitis (60-90% of cases); 2) perinuclear ANCA (pANCA), which are more common in MPA (50-80% of patients) and less common in Crohn's disease, eosinophilic granulomatosis with polyangiitis (EGPA) (Cherdja – Strauss syndrome), NP, ulcerative colitis. Perinuclear glow is associated with MPO [7].

In the pathogenesis of ANCA-associated SV the leading role is played by the activation of the complement system by an alternative pathway with the formation of C5a, the interaction of C5a and C5aR. It leads to neutrophil degranulation [8, 9], to the secretion of anti-MRONG / anti-PR3NG, which damages the vascular endothelium of the microcirculatory bed, mainly in the skin, lungs and kidneys [10].

Microscopic polyangiitis (MPA) – necrotizing vasculitis with minimal or no immune deposits, with predominant vascular lesions of the microcirculatory bed (capillaries, venules, arterioles), rarely – small and medium-sized arteries. In this case, the clinical picture is dominated by the phenomena of necrotizing glomerulonephritis, skin lesions, rarely – pulmonary capillaries. It is noteworthy that pulmonary embolism of the pulmonary artery MPA is found 10 times more often than in classical nodular polyarteritis (NP) and twice more often than in granulomatous polyangiitis (GPA) (in the past – Wegener's disease) [11,12].

The diagnosis of MPA is based on complaints, clinical, immunological and morphological examination data. It is well known that diagnostic criteria are: fever, malaise, weight loss; arthritis, myalgia; changes in the kidney (often a rapidly progressing glomerulonephritis); skin manifestations in the form of erythema; abdominal syndrome, bleeding diarrhea; mono- or polyneuropathy; presence of pulmonary infiltrates (often with fatal bleeding) and detection of perinuclear autoantibodies to cytoplasmic components of neutrophils, antimyeloperoxidase in serum.

Renal impairment in MPA is manifested in the form of rapidly progressing glomerulonephritis with proteinuria (sometimes with nephrotic syndrome), micro- or hematuria, early renal failure (RF). Often patients are concerned cough, chest pain, sometimes – hemoptysis and pulmonary hemorrhage. In this case, radiographs of the lungs find infiltrative changes without decay sites, often with the development of pleurisy. In laboratory examination patients have moderate hypochromic anemia, neutrophilic leukocytosis, acceleration of erythrocyte sedimentation rate (ESR), increased levels of C-reactive protein (CRP) and creatinine.

According to the literature, the course of MPA has individual characteristicsHowever, the frequency of clinical symptoms with MPA varies. Myalgia, arthritis, arthralgia occur in 30-65%; purpura or erythema, contact bleeding – 40-58%, abdominal pain – 30-58%, lung damage – 57-100%, gastrointestinal bleeding – 20 – 30%, lesions of the eyes and ENT organs – in 30%, fever – 50%, arterial hypertension – 40-50%, isolated urinary – 70-90%, of which 50% of patients require renal replacement therapy (RRT). In 75% of patients exhibit high titer of pANCA/MPO [13, 14].

The difficulty of diagnosis is the need for a simple differential diagnosis, through a similar clinical picture, even within the ANCA-associated systemic vasculitis, the need for histopathological examination of skin, kidney or lung biopsy, the useing of not always available immunological tests.

ANCA – testing must be performed in accredited laboratories participating in quality assurance testing programs. In all cases, it is necessary to perform a biopsy of the kidney, which is the most reliable for establishing the nature and prevalence of inflammation, diagnosis and determination of vasculitis [15].

Renal lesions are typical of all ANCA-associated vasculitis, but the incidence of nephropathy is different: maximum (90%) with MPA and GPA (90%), rarely – with EGPA (20-45%). As a rule, kidney damage in MPA is accompanied by a sharp onset and a more aggressive course. It is essential to diagnose the disease as soon as possible and to start treatment in a timely manner. But in a third of patients do not detect antibodies at all, that is, a negative ANCA result does not rule out vasculitis in the presence of clinical signs of active disease [16].

The urgency of the problem is increasing due to the emergence of a certain tendency to increase these diseases.

CASE REPORT

THE CLINICAL CASE №1

Patient C., 52 years old, was admitted to the center of nephrology and dialysis of the Poltava Regional Clinical Hospital of N. Sklifosovsky (PRCH) 29/07/2019. At the time of hospitalization, she complained of shortness of breath with little exercise, increasing blood pressure (BP) to 150/100 mm Hg., dry mouth, general weakness. There was no history of kidney or lung disease, however, during the 6 months before the first clinical symptoms, there was a periodic increase in blood pressure up to 150/100 mm Hg. Considers herself sick since April 2017, when after eating fresh vegetables, she felt acute pain in the right half of her stomach. She was treated on her own, the condition improved, but a week later there was an attack of acute pain in the left half of the abdomen, noted urinary opacity. Subsequently, there were complaints of frequent urination at night (5-6 times), nausea, headache in the temporal area, dry mouth, fever to subfebrile numbers. From 05/05/2017 to 11/05/2017 were examined and treated inpatient at the place of residence with suspected secondary (paraneoplastic?) nephropathy. 07/05/2017 consulted by oncologist – oncology excluded, diagnosed with small uterine fibroids. General clinical examinations were performed, which revealed significant deviations: in the general analysis of urine (GBA) – proteinuria (0.158 – 1.79 g / l), leukocyturia (by ½ in the field of view (f/v)), erythrocyturia (12- 14 in f/v); in biochemical analysis of blood (BAB) – azotemia (increase of creatinine level to 438 μmol / l). The glomerulonephritis with acute kidney damage was diagnosed. Symptomatic treatment did not give effect.

Given the above complaints, changes in the results of analyzes, ineffectiveness of treatment, on May 11, 2017, the patient was referred for consultation to a nephrologist of PRCH, where she was hospitalized to the center of nephrology and dialysis for examination to clarify the diagnosis and treatment.

According to the analysis of medical records, changes in the objective status of the patient were noted: pallor of the skin, puffiness of the face, displacement of the left border of cardiac dullness by 1.5 cm outside the left mid-clavicular line, symptom of tapping moderately positive on both sides. In the general analysis of blood (GBA) – leukocytosis ($12x10^9$/l), anemia (Hb – 107 g/l, erythrocytes –$3,47x10^{12}$/l), acceleration of the erythrocyte sedimentation rate (ESR) – up to 34 mm/h; in the GUA there were signs of urinary syndrome (erythrocyturia (unchanged erythrocytes – for all f/v), leukocyturia (8-10 in f/v), cylindruria (hyaline 2-3 in f/v). In the urine analysis for Nechiporenko revealed erythrocyturia. Daily proteinuria (DP) was 1.4 g/d. At the same time, the level of creatinine and urea in the BBA increased to 505 μmol/l and 17.8 mmol/l, respectively, and the glomerular plasma filtration rate (PFR) according to the SKD-ERI formula was 11 ml/ min./1,73м^2. On the electrocardiogram (ECG) – deviation of the electric axis to the left, signs of blockage of the anterior branch of the left leg of the bundle branch, changes of the myocardium of the anterior-membranous area. Ultrasound examination of abdominal organs showed an increase in ultrasound echogenicity of the parenchyma of both kidneys with the presence of symptoms hypoechoic pyramids and reduction of parenchyma thickness of the right kidney up to 1.4 cm, left – up to 1.2 cm. Consulted by urologist, neurologist. Diagnosed cervical cystitis, dyscirculatory encephalopathy (hypertensive-dyshormonal) I st., with vegetative-vascular dysfunction.

Membrane stabilizing, detoxifying, anticoagulant, antihypertensive and anti-anemic therapy did not produce a pronounced effect. In the dynamics of laboratory param-

eters from 22/05/17, normalization of the indicators was noted, however, anemia progressed, ESR accelerated to 46 mm/h, creatinine and urea levels increased to 738.7 μmol/l and 22.5 mmol/l, respectively. DP – in the range: 1.4 – 1.1 g/d. 22/05/17 diagnosed with acute rapidly progressing glomerulonephritis, urinary syndrome. Taking into account the patient's complaints, the presence of changes in the results of laboratory and instrumental examinations indicated the ineffectiveness of the therapy and the high probability of the patient having systemic vasculitis with renal injury. In order to verify the diagnosis, it was recommended to send for consultation to the State Institution «Institute of Nephrology of the National Academy of Medical Sciences of Ukraine», where on May 24, 2017, the patient was consulted by a nephrologist, but refused the proposed nephrobiopsy..

31/05/2017 with complaints of intense pain in the lower leg muscles, shortness of breath, low physical activity, increased blood pressure, pronounced general weakness, the patient was re-admitted to the center of nephrology and dialysis. At the examination from 01/06/2017: in GBA – there were signs of anemia (er. – $3{,}0x10^{12}$ /l, Hb – 84 g / l), accelerated ESR – 42 mm/h, normal level of leukocyte indexes ($6{,}6x10^{9}$/l) on the background of shift of leukocyte formula to the left %; in BAB – azotemia (creatinine – 1161.8 μmol/l, urea – 35.0 mmol/l); the GUA retained the above-mentioned signs of urinary syndrome. Intensive detoxification, membrane stabilizing, anti-anemic and antihypertensive therapy was carried out. After a two-week course of treatment, the results of GBA showed a slowing of ESR to 20 mm / h, but there were signs of anemia (er. – $2{,}47 \cdot 10^{12}$/l, Hb – 73 g/l), leukocytosis ($11{,}1 \cdot 10^{9}$ /l) and lymphocytopenia (9%); there were no significant dynamics in BBA in the levels of creatinine and urea (1140 μmol / l and 33.7 mmol / l, respectively), hyperkalaemia (6.04 mmol / l) was first detected. DP – 1,63 g / d., DD – 1,5 l.

Data from the immunological examination of the patient from 06/02/17: increased titer of AT IgG ANCA to 1: 3200 (<1: 100 – negative result) and high-positive result of ANCA to MRO (6.1 R) (up to 1.0 – negative, more than 5.0 – highly positive result). Thus, the results of the study could be interpreted as verified systemic ANCA (MPA +) vasculitis.

Due to the presence of arterial hypertension, history of abdominal syndrome, rapidly progressing GN with the development of RF, anemia, elevated titer of AT IgG ANCA and ANCA to MRO by decision of the council of 08.06.2017 was diagnosed with: ANCA (MPO +) vasculitis with preference kidney. It is recommended to change the tactics of treatment of the patient. Methylprednisolone was administered at a dose of 32 mg / day and endoxan 50 mg / day, orally, in combination with NRT by acute hemodialysis (AHD). Fifteen days of combination therapy improved the patient's well-being, tended to decrease serum creatinine and urea (276 μmol / l and 11 mmol / l, respectively). The patient was advised to continue pathogenetic therapy at the above dose and to further carry out NRT by programmatic hemodialysis (PHD). However, due to the reduction of the diameter of the vein (1.0 mm) and artery (1.2 mm), the establishment of arterio-venous (AV) fistula proved impossible. Therefore, on June 23, 2017, the patient was consulted at the Institute of Surgery and Transplantology O.O. Shalimov, superimposed arterio-venous alosunt. Since June 27, 2017, the PGD has been started.

The six-month course of the above therapy led to an improvement in the patient's general, positive dynamics in laboratory parameters (serum creatinine: 250 – 210 – 180 μmol / l). Due to the effectiveness of the treatment, 01/01/2018, PGD sessions and cytostatics were canceled. The patient is recommended to continue taking medrol, at a dose of 32 mg / day, orally, with a gradual reduction of the dose by 2 mg every 2 weeks, until the minimum effective maintenance dose will be reached. At the time of discharge from the hospital, there was a decrease in ESR up to 16 mm / h, an increase in Hb up to 93 g / l, serum creatinine levels stabilized at 150 μmol / l.

Since then, the patient has felt satisfactory, the dose of methylprednisolone was gradually reduced to 4 mg / day, and from May 2019 completely stopped taking it..

In July 2019, after suffering SARS, the patient developed shortness of breath with little exercise, dry mouth, blood pressure was maintained at 160/100 mm Hg. against antihypertensive therapy, a general weakness. The examination revealed azotemia (creatinine 800 μmol / l). From 25/07/19 to 28/07/19 – re-hospitalization in therapeutic department at the place of residence. Evaluation of the results of laboratory tests revealed significant changes in indicators: in GBA – signs of progression of anemia (er. – $2.7 \cdot 10^{12}$ / l, Hb – 75 g / l), acceleration of ESR (19 mm / h); in BBA – expressed azotemia (creatinine – 902,6 μmol / l, urea – 26,8 mmol / l); in GUA – proteinuria (2.96 g / l). Symptomatic therapy was done. 29/07/19 was consulted by the regional rheumatologist and nephrologist. Diagnosed with: Systemic vasculitis: microscopic polyangiitis, stage II activity with kidney damage. CKD V: glomerulonephritis, nephrotic syndrome. Stage II hypertension, grade 3, the risk is very high. On the same day she was transferred to the Center for Nephrology and Dialysis of PRCH for further treatment.

Laboratory indicators at hospitalization: reduction of erythrocytes is revealed (2.8 × 1012 / l) and Hb (72 g / l), lymphocytes (14%); acceleration of ESR (17 mm / h); increase in creatinine (964.8 μmol / l) and urea (33.7 mmol / l). ECG data from 07/30/19: sinus rhythm, heart rate – 75 / min, electrical axis of the heart is rejected to the left. Reduced QRS voltage in standard and amplified leads, V1-V2. Blockage of the anterior branch of the left leg of the bundle branch. Ischemic type of repolarization disorders. Data fibrogastroduodenoscopy (FGDS) from 31/07/19. Conclusion: – chronic duodenal ulcer in remission. On rheoencephalography from 01/08/19, the hemispherical asymmetry (D> S) was detected; the volume blood flow of the right hemisphere is sufficient, the left is reduced; increased peripheral vascular resistance, arterial tone is unstable. Data of ultrasound from 01/08/19. Conclusion: presence of a small amount of free fluid in the pleural

cavities (in the right pleural cavity – ≈ V 40 ml, in the left ≈ V 15 ml), ultrasound – signs of nephritis, progression of nephrosclerosis (reduction of the size of the kidneys (right – up to 7,9x3,2 cm) , left – up to 8.0x4.4 cm) and thickness of their parenchyma (1.0 cm and 1.3 cm, respectively). Echocardiography (Echocardiography) of 02/08/19 showed a decrease in myocardial contractility, signs of left ventricular hypertrophy with type II diastolic dysfunction; enlargement of the cavities of both atria, compaction of the aorta, aortic valve; insufficiency of the mitral valve of the II degree, relative insufficiency of the tricuspid valve of the II degree, pulmonary hypertension of the I-II degree.

Consulted by related specialists: rheumatologist, hematologist, gastroenterologist. Diagnosed with: systemic vasculitis: microscopic polyangiitis, grade III activity. CKD V: rapidly progressing glomerulonephritis, urinary syndrome. Stage II hypertension, grade 3, the risk is very high. Secondary normochromic anemia of moderate severity. Peptic ulcer of the duodenum with deformity of the bulb, with high acid-forming gastric function in remission.

Treatment: Re-initiation of NRT sessions using PGD mode: 3 times a week for 4 hours on the background of pathogenetic (given the high degree of process activity) therapy. After a two-week course of prescribed therapy, there was a positive trend in creatinine (962.5 – 946.2 – 709.9 mmol / l) and urea (36.4 – 34.8 – 26.5 mmol / l) in serum, and PD decreased up to 2.73 g / d. 08/16/19 The patient was transferred for further treatment to the therapeutic department, at the place of residence.

THE CLINICAL CASE №2

Patient M., 48 years old, hospitalized for examination and correction of treatment at the Nephrology and Dialysis Center in November 2019 with complaints of periodic lumbar pain, general weakness.

From the anamnesis it is known that the patient considers herself ill since 2012, when after physical exertion she felt pain in the joints, especially – in the left knee joint. She consulted a family doctor at the place of residence, consulted by a traumatologist. Diagnosis: osteoarthritis of the left knee. Prescribed anti-inflammatory and chondroprotective therapy did not show a pronounced effect. In connection with the expressed pain syndrome, the patient went to a consultation at the State Institution «Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine», where the diagnosis was confirmed and it was found that the joint was preserved, the patient did not need surgery. Recommended: Mucosate intramuscularly, according to the scheme for 6 months. From 2012 to 2013 the patient periodically received courses of non-steroidal anti-inflammatory drugs and chondroprotective agents. The severity of the pain in the joints decreased. However, since then, the patient has begun to notice an increase in blood pressure up to 160/100 mm Hg.

For four years the patient lost 15 kg. In the fall of 2016, symptoms of polyarthritis began to increase and the patient began to notice pain in the lumbar region, a change in the color of urine (such as «meat washes») against the background of fever to subfebrile numbers, and in parallel increased general weakness. After the examination at the place of residence, glomerulonephritis was diagnosed. To clarify the diagnosis and the purpose of treatment, the patient was hospitalized at the therapeutic department of the central district hospital of Lubny. Biochemical examination of the blood revealed for the first time signs of impaired renal function (blood creatinine level – 210 μmol / l), in the GUA – signs of urinary syndrome (proteinuria – 1,13 g / l and erythrocyturia (changed er. – by ½ f/v.). Appointed detoxification, antihypertensive and antibacterial therapy. After treatment, the pain in the joints decreased, but continued to be disturbed by the aching pain in the lumbar region, the creatinine levels increased to 400 μmol / l, and proteinuria and erythrocyturia were constantly detected in the urine. Consulted by a PRCH nephrologist and hospitalized at the Nephrology and Dialysis Center with a diagnosis of CKD II: glomerulonephritis, urinary syndrome, exacerbation. CKD I degree. Stage II hypertension, 2 degrees, risk is high.

At the time of admission, objective examination revealed swelling on the face, the shins, signs of left knee arthritis (painful on palpation, swelling, restriction of active and passive movements). The left border of cardiac dullness is displaced 1 cm outside the midclavicular line. Auscultatory – heart sounds are audible, short systolic murmur over apex, accent of II tone over aorta. BP – 150/75 mm Hg. (against the background of continuous antihypertensive therapy). The symptom of tapping is positive on both sides. DD – 3,0 l. At laboratory examination – signs of anemia of moderate severity, thrombocytosis, acceleration of ESR up to 68 mm / h .; in GUA – proteinuria (protein – 1.13 g / l), erythrocyturia (altered er. by 1/2 f/v., unchanged 10-20 in f/v.), moderate leukocyturia, cylindruria (hyaline 3-4, single granular and waxy in f/v), bacteriuria; elevated levels of creatinine and urea (268 μmol / l and 12.5 mmol / l, respectively) were maintained in the BBA. The speed of CFR according to the formula SKD-ERI was 54.9 ml / min / 1.73 m^2.

During the hospital stay the patient is thoroughly examined. On the chest Ro-gram – the pulmonary fields are transparent, in the basal departments of the lungs the pulmonary pattern is thickened and deformed, the roots are fibrous, the right sinus is free, the left is obliterated, massive left-sided pleural-diaphragmatic quadruplets. On the Ro-gram of the left knee joint in 2 projections – signs of osteoarthritis II st. Ultrasound revealed ultrasound – signs of nephritis, gallstones, moderate enlargement of the liver. Computed tomography (CT) CT data: CT revealed signs of decreased renal parenchyma density, small pelvic effusion, moderate hepatomegaly, pelvic lymphadenopathy. FGDS revealed congestive gastropathy; at fibrocolonoscopy – chronic left-sided colitis. When sowing urine on the microflora enterobacter 10^4 CFU. The performed sternal puncture of changes did not reveal. Immunological examination of blood for antibodies to double-stranded DNA, AB IgG showed a negative result. Consulted neurologist,

hematologist, gynecologist, gastroenterologist, pulmonologist, rheumatologist. The following diagnosis was established: CKD II: glomerulonephritis, urinary syndrome, exacerbation stage. CRF I degree. Stage II hypertension, degree 2, risk is high. Dysmetabolic encephalopathy of II degree with bilateral reflex-pyramidal insufficiency, moderate vestibulo-atactic syndrome, cephalic syndrome. Hypochromic anemia of unspecified genesis, thrombocytosis. Chronic gastroduodenitis in the acute stage. Duodenogastric reflux. Gallbladder dysfunction. Osteoarthritis: Stage II gonarthritis with reactive synovitis. FJI II. Appointed detoxification, membrane stabilizing, anticoagulant antihypertensive therapy, topical anti-inflammatory drugs.

The lack of positive dynamics in laboratory parameters is noteworthy. In the clinical picture, signs of glomerulonephritis (proteinuria – 0.79 – 0.5 g / l), erythrocyturia (altered erythrocytes by 1/2 – 1/4 f/v.), Moderate leukocyturia, cylindruria prevailed; in Nechiporenko urine analysis – leukocyturia (14250 in 1 ml), erythrocyturia (20000 in 1 ml), cylindruria (187 in 1 ml); DP – 1,62 g / d., In BBA – creatinine – 283 μmol / l, urea – 15,8 mmol, in the urine analysis according to Zimnitsky – hypoisostenuria, anemia (er. – $3,2x10^{12}$ / l, Hb – 73 g / l) and signs of systemic inflammation (moderate leukocytosis, ESR acceleration up to 73 mm / h.).

Due to the lack of positive effect of treatment, a consultation at the State Institution of the "Institute of Nephrology of the National Academy of Medical Sciences of Ukraine" is recommended, which the patient categorically refused.

She has since continued treatment at the place of residence. Three months after discharge from the hospital, the patient again experienced pain in the lumbar region, noted reddening of the urine, swelling in the face and legs increased, pain in the left knee joint increased. At the same time, the blood pressure periodically increased to 180/110 mm Hg. She was re-treated at the Center for Nephrology and Dialysis of PRCH. On physical examination: swelling of the left knee joint, active and passive movements are limited due to pain. Otherwise, there were no significant dynamics. Laboratory parameters still showed moderate anemia, moderate eosinophilia – 6-7%, ESR acceleration – 62-68 mm / h, signs of renal dysfunction (creatinine – 142 μmol / l) and urinary syndrome (proteinuria) – 0,365 g / l -1,62 g / d., eErythrocyturia (altered erythrocytes 20-35 in f/v, cylindruria (60 in 1 ml)). On the Ro-gram of the left knee joint in 2 projections – signs of osteoarthrosis II degree. After the course of treatment the patient was discharged in satisfactory condition.

However, in September 2017, the patient's condition worsened significantly: redness of the sclera of the left eye appeared, dizziness developed, moderate pain began in the epigastric region. Consulted by an optometrist and diagnosed with left eye uveitis. Outpatient treatment did not give effect. Due to the further deterioration of the condition, she was hospitalized at the center of nephrology and dialysis. During the treatment there was a slight improvement in clinical and laboratory parameters. GBA increased the number of red blood cells ($4.01x10^{12}$ / l) and Hb (106 – 111 g / l), slowed ESR (50 – 37 mm / h), but no significant changes occurred in the GUA. Nitrogen metabolism was normalized in the BBA (creatinine – 101 μmol / l, urea – 6.2 mmol / l), other indicators also within normal values. The rate of GF is 85.1 ml / min / 1.73 m3; tubular reabsorption (TR) – 95.1%. Acute-phase blood counts: rheumatoid factor, antistreptolysin-O – negative, but C-reactive protein was positive – 8.6 mg / l. According to ultrasound: right kidney 11,8x3,7 cm, parenchyma – 0,9 cm, cup-bowl complex compact, partially doubled, parenchyma moderately enhanced, microliths; left kidney – 9,1x4,0 cm, parenchyma – 1,1 cm, cup-bowl complex is structurally preserved, parenchyma moderately enhanced, microliths. Conclusion: ultrasound – signs of nephritis. Microliths in the kidneys. Pathology of the liver, spleen, pancreas was not detected.

Consulted by an ophthalmologist (09/22/17, 09/25/17, 09/26/17): Diagnosis: Left eye uveitis. Secondary (post uveal) glaucoma OS.

Given the weight loss of the patient, the presence of hypertension, glomerulonephritis with renal failure, recurrent uveitis, anemia, increased content of C-reactive protein, inefficiency of symptomatic drug therapy in the patient was suspected presence of systemic vasculitis. Immunological examination of the patient's blood was performed to verify the diagnosis.

Increased titers of IgG antibodies to MPO> 8 (<1.0 – negative) and antibodies to double-stranded DNA were detected. To clarify the diagnosis, the patient was referred to the State Institution «Institute of Nephrology NAMS of Ukraine» for the purpose of nephrobiopsy. However, the patient flatly refused and was discharged to continue outpatient treatment at the place of residence.

From February to November 2018, the patient was repeatedly treated by an ophthalmologist with a diagnosis of slow-moving uveitis, secondary glaucoma, complicated by cataracts of the left eye. Pterigium I degree of both eyes – there was no effect. It was periodically inspected at the place of residence. As a result of clinical research methods leukocytosis, acceleration of ESR (45-68 mm / h), mild anemia, changes in urine (proteinuria – 0.46-0.95 g / l), erythrocyturia (unchanged er. – 25-35 in f/v were preserved), leukocyturia (10-6 in f/v). In the analysis of urine according to Zymnitsky: specific gravity of urine – 1005-1012, DD – 2650 ml. She was treated at the place of residence, repeatedly consulted by a PRCH nephrologist.

In November 2018, the patient's condition suddenly worsened: growing shortness of breath, pain in the lumbar region, frequent urination. She was referred to a nephrologist and re-admitted to a Nephrology and Dialysis Center.

In the objective status, compared with the data of the previous hospitalization, a pronounced deformity of the left knee joint, sharp restrictions of active and passive movements in the left knee due to the pronounced pain syndrome, attracted attention. The results of laboratory examinations revealed signs of renal dysfunction (creatinine – 172 μmol / l, urea – 10 mmol / l, GF rate was 72.8 ml / min / 1.73m3, KR – 93.1%); urinary syndrome (trace

proteinuria, erythrocyturia (1200 in 1 ml), mild anemia and increase in cholesterol (CL) up to 7.79 mmol / l. Ultrasound: signs of increased echogenicity of the renal parenchyma. Symptomatic treatment was performed.

The presence of hypertension, weight loss, eye damage (uveitis), muscle damage (myalgia), joint damage (osteoarthritis), anemia, constant changes in the urine (erythrocyturia, proteinuria), increased creatinine, and repeated confirmation of increased levels of antibodies to MPO (> 8) allowed to make a diagnosis: Systemic vasculitis: microscopic polyangiitis with renal involvement. Stage II hypertension, degree 2, high risk. CHD: diffuse cardiosclerosis. CHF 0 degree. FC I. Hypercholesterolemia.

She was consulted by a nephrologist at the Institute of Nephrology of the National Academy of Medical Sciences of Ukraine, and a rheumatologist from the State Institution of Cardiology. An additional immunological blood test showed positive blood test results p-ANCA (perinuclear) 1:40 (reference values 1:<10). Diagnosed with ANCA (MPO+) vasculitis with predominant renal involvement. Pathogenetic therapy was prescribed. Since then, the patient has been receiving endoxan 500 mg and methylprednisolone 12 mg daily (oral), telmisartan under the control of BP.

In October 2019 she was consulted by a rheumatologist of the State Institution «Institute of Cardiology named after academician M.D. Strazhesko»of the National Academy of Medical Sciences of Ukraine. The patient is advised to reduce the dose of methylprednisolone to 10 mg / d.

In November 2019, the patient again noted a change in the color of urine to «red», a general weakness. The examination revealed proteinuria – 1.0 g / l, in which case the patient was re-hospitalized to the center of nephrology and dialysis of PRCH for examination and correction of treatment.

On initial physical examination: skin and mucous membranes without features. Peripheral lymph nodes are not enlarged. Deformation of the left knee joint with restriction of active and passive movements. Moderate edema around the articular tissue of the left knee. Swelling of the face. The left border of cardiac dullness is displaced outward from the middle-clavicular line by 1 cm. Auscultative: heart tones are audible, short systolic murmur over apex, accent II tone over aorta. BP up to 140/100 mm Hg.

At examination: proteinuria (protein – 0,174 g / l), unchanged erythrocytes – 3-8 in f/v., Leukocytes – 0-2; in the analysis of urine by Nechiporenko: erythrocyturia – 7800 in 1 ml; in the urine analysis according to Zymnitsky – hypostenuria (specific gravity 1004-1011), DD – 1500 ml, nocturia. DP – 0,342 g / day. In the BBA – increase of creatinine level to 134 μmol / l, CL – 7,83 mmol / l, triglycerides – 2,75 mmol / l; atherogenic index – 3.5. In GBA: ESR – 13 mm / h, leukocytes – $4{,}93x10^9$ / l, lymphocytopenia (10%), Hb – 120 g / l, erythrocytes – $3{,}90x10^{12}$ / l, moderate thrombocytosis (334x109 / l). The patient received prednisolone 10 mg, azathioprine 100 mg / d., Orally, in the morning, after meals and symptomatic (antihypertensive, detoxification, nephroprotective) therapy.

As a result of the treatment, the patient's condition improved significantly, positive dynamics in laboratory parameters were noted: the level of creatinine in the BBA decreased to 101 μmol / l, CL – to 6.4 mmol / l. In GUA – proteinuria – 0.04 g / l, ep. – 2 – 3 in f/v, in Nechiporenko urine analysis: erythrocyturia decreased to 2540 / ml. It is recommended to continue pathogenetic therapy with azathioprine 100 mg / d, with a gradual decrease in the dose of methylprednisolone per ¼ tablet once every two weeks to 5 mg / d.

Microscopic polyangiitis is a rare necrotizing systemic vasculitis without immunoglobulin deposits associated with antineutrophil cytoplasmic antibodies. In the overwhelming majority of cases is characterized by lesions of the vessels of the microcirculatory bed, but there is a possibility of lesions of the arteries of small and medium diameter.

Usually manifestation of the disease begins with pulmonary-renal syndrome, namely: hemorrhagic alveolitis and necrotizing glomerulonephritis. However, the analysis of modern literature shows that it may have a very diverse beginning, different variants of the course with symptoms of damage to different organs that are characteristic of other SV. Practice shows that MPA is quite common in the practice of nephrologists.

It should be clearly understood that MPA is considered a fatal pathology. An important factor in determining the prognosis and quality of life of such patients is the timeliness of diagnosis, a clear differential diagnosis and the adequacy of medical care.

The difficulty of verification lies in the lack of clear classification criteria, the need for more extensive use of modern histological examination methods, with the mandatory use of immunological tests, which are not always available yet. Modern aspects of diagnostics include analysis of clinical manifestations, positive results of immunological studies of serum for detecting perinuclear autoantibodies to cytoplasmic components of neutrophils, antimyeloperoxidase in serum, antibodies to MPO-ANCA, histopathological examination of skin, kidney or lung biopsy specimen, signs of systemic inflammation and anemia in blood test, laboratory signs of rapidly progressing glomerulonephritis with development of renal failure.

As can be seen from a detailed chronological analysis of the course of MPA in the first case, we can state the gradual rate of progression of the disease with the development of such nonspecific symptoms as: increase in blood pressure, abdominal syndrome, fever to subfebrile numbers, nausea, dry mouth, headache, all of which were accompanied by symptoms suggestive of kidney damage, which were initially considered as glomerulonephritis, complicated by acute kidney damage. Differential diagnosis was made within paraneoplastic nephropathy and systemic vasculitis.

Ineffectiveness of the conducted symptomatic, membrane-stabilizing therapy, presence of arterial hypertension (AH), signs of systemic inflammation, anemia in the general blood test, proteinuria, hematuria, azotemia, positive results of immunological studies of serum for the diagnosis

of MPA vasculitis: microscopic polyangiitis, grade III activity, with kidney damage.

The appointment of patients with methylprednisolone at a dose of 32 mg / day and endoxane 50 mg / day, orally, in combination with the KRT method of acute, and further – program hemodialysis not only led to the control of the symptoms of the disease, but also allowed to renal replacement therapy stop taking cytostatics and gradually reduce glucocorticosteroid intake. This indicated the selection of the optimal treatment strategy. However, the unauthorized discontinuation of glucocorticosteroids in the future provoked a relapse and led to the need to resume the course of the above therapy.

In the second clinical case, concomitant joint lesions came to the fore, so the diagnostic search was initially aimed at clarifying the nature of joint syndrome and its treatment. However, weight loss, the symptoms of glomerulonephritis, which occurred only four years after the first signs of arthritis, and then – the presence of hypertension, eye damage (uveitis), muscle damage (myalgia), azotemia, anemia on the background of joint damage (osteoarthritis) suspect systemic vasculitis. Repeated positive blood test results for antibodies to MPO (>8) and p-ANCA (1:40) allowed to make the diagnosis: Microscopic polyangiitis with kidney damage. Pathogenetic therapy only (methylprednisolone 12 mg and endoxane 500 mg daily) for eleven months, followed by administration of methylprednisolone 10 mg and azathioprine 100 mg / d. led to a significant improvement in the patient's overall condition, a positive trend in laboratory parameters, which prompted nephrologists to gradually reduce the glucocorticosteroid dose to a minimum effective.

The following clinical examples demonstrate to physicians that systemic vasculitis is quite common in the practice of nephrologists.

CONCLUSIONS

Thus, the described clinical cases differ slightly in features of the debut and the course of the disease, but they also have common features, which were manifested by renal involvement with the development of renal failure, arterial hypertension and anemia.

Therefore, before deciding on the primary or secondary nature of glomerulonephritis, nephrologists need to carefully evaluate the entire complex of objective and subjective features, laboratory and instrumental examination data, identify symptoms of multiple organ lesions, signs of disease progression.

In the case of the detection of additional signs that may indicate systemic pathology, it is always advisable to consider the possibility of the presence of kidney damage in patients, as a symptom of another disease, which is accompanied by similar symptoms, especially systemic vasculitis. Moreover, it is extremely important to be able to recognize them in time and to send these patients for further immunological examination and for nephrobiopsy, since the prospects of effective treatment and quality of life will depend on this.

Thus, clinical examples demonstrate to physicians that systemic vasculitis can often hide under the «mask» of other diseases and require timely diagnosis and immediate pathogenetic treatment.

REFERENCES

1. Holle J.U. Seropositive and negative ANCA-asso ciated vasculitis, anti-MPO and PR3-vasculitis: Different outcomes? La Presse Médicale. 2013;42(4P2):616–619.
2. Cornec D., Cornec-Le Gall E., Fervenza F.C.et al. ANCA-associated vasculitis [mdash] clinical utility of us– ing ANCA specificity to classify patients. Nat. Rev. Rheumatol. 2016;35:953–960.
3. KDIGO 2012. Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney International Supplement 2013;3:1–150.
4. Takahashi M., Otsubo S., Takei T. et al. Anti-glomerular basement membrane antibody disease with granulomatous lesions on renal biopsy. Internal Medicine. 2007;46(6):295-301.
5. Scott D.G.I., Watts R.A. Epidemiology and clinical features of systemic vasculitis. Clin. Exp. Nephrol. 2013;17:607-610.
6. Katerenchuk I.P., Tkachenko L. A.,Yarmola T.I. et al. Churg-Strauss syndrome: clinicalcaseand. Wiad. Lek. 2019;72(4):723-726.
7. Katerechuk I.P., Tkachenko L.A., Yarmola T.I. Urazhennia nyrok pry revmatychnykh zakhvoriuvanniakh [Kidney damage in rheumatic diseases]. K. Medknyha. 2017: 55-99. (In Ukrainian).
8. Reumaux D., Kuijpers T., Hordijk P.L. et al. Involvement of Fcy receptos and B2 integrins in neutrophil activation by antiproteinase-3 or anti-myeloperoxidase antibodies. Chn. Exp. Immunol. 2003;134:344 – 350.
9. Chen M. Kallenberg CY ANCA –associated vasculitides – advances in pathogenesis and treatment. Nat Rew Reumatol. 2010;6:653-664.
10. Yates M., Watts R.A., Bajema I.M. et al. EULAR/ ERA-EDTA recommendations for the management of ANCA-associated vasculitis. Ann. Rheum. Dis. 2016;75:1583–1594.
11. Bakkaloglu A., Ozen S. Wegener Granulomatosis, Microscopic Poliangiitis and Childhood Poliarteriitis Nodosa. Comprehensive Pediatric Nephrology. Copiring. Mosby. 2008; 353 -358.
12. Vamvakopoulos J., Savage C., Harper L. ANCA-associated vasculitides in children –lessons from the adult literature. Pediatr Nephrol. 2010;25:1397-1407.
13. Ozen S. Vasculitis. Pediatric Nephrology. 6- th edition. IPNA. Springer-Verlag. 2009;2:1001-1009.
14. Vanoni F., Bettinelli A., Keller G. et al. Vasculitides associated with IgG antineutrophil cytoplasmic autoantibodies in childhood. Ped Nephrol. 2010;25: 205-212.
15. Legendre P., Régent A., Thiebault M., Mouthon L. Anti-Endothelial Cell Antibodies in Vasculitis: A Systematic Review. Autoimmun. Rev. 2016;16(2):146–153.
16. Haubitz V. ANCA-associated vasculitis: diagnosis, clinical characteristics and treatment. Vasa. 2007;36:81-89.

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ORCID and contributionship:
Ivan P. Katerenchuk: 0000-0003-3765-4895 [A,B,E]
Lydia A. Tkachenko: 0000-0001-9356-6385 [A,B,F]
Tatiana I. Yarmola: 0000-0002-7428-0223 [A,C]
Victoria V. Talash: 0000-0002-5700-557X [A,C,D]

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CORRESPONDING AUTHOR
Tatiana I. Yarmola
Ukrainian Medical Stomatological Academy
23 Shevchenko st., 36024 Poltava, Ukraine,
tel:+380505667005
e-mail: tyarmola@gmail.com

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CASE STUDY

GLYCOGEN STORAGE DISEASE TYPE II: A NARRATIVE LITERATURE REVIEW AND A CASE REPORT OF LATE-ONSET POMPE DISEASE IN A YOUNG WHITE CHILD

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Hanna V. Palahuta[1], Olena Y. Fartushna[2], Olha G. Selina[2], Yevhen M. Fartushnyi[2], Tetiana V. Koval[3]
[1]STATE UNIVERSITY "UZHHOROD NATIONAL UNIVERSITY", REGIONAL CLINICAL CENTER OF NEUROSURGERY AND NEUROLOGY, UZHHOROD, UKRAINE
[2]UKRAINIAN MILITARY MEDICAL ACADEMY, KYIV, UKRAINE
[3]STATE UNIVERSITY "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE

ABSTRACT

At all ages, skeletal muscle weakness characterizes Pompe disease, causes mobility problems and affects the respiratory system. We aimed to provide a narrative review of terminology, etiology, epidemiology, clinical manifestations, complications, and prognosis of Pompe disease, supported with a clinical case presentation.

The clinical manifestation and complications of Pompe disease are illustrated with the clinical case presentation of a late-onset form in a white child. A comprehensive electronic literature search was performed on Ovid, Google Scholar, Scopus, PubMed, Embase, Cochrane Database, and World Health Organization databases to identify the articles that discussed Pompe disease.

KEY WORDS: Glycogen storage disease, Pompe disease, review, clinical case, late-onset

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INTRODUCTION

Glycogen storage disease (GSD) is a metabolic disorder caused by enzyme deficiencies affecting either glycogen synthesis, glycogen breakdown, or glycolysis (glucose breakdown), typically in muscles and/or liver cells. At least 15 types of GSD have been identified, all resulting in abnormal glycogen metabolism and an accumulation of glycogen in these cells. [1, 2]

The main types of GSD are categorized by number and name, as follows:

- Type I (Von Gierke disease; the most common type that accounts for 90% of all glycogen storage disease cases);
- Type II (Pompe's disease, acid maltase deficiency);
- Type III (Cori's disease);
- Type IV (Andersen's disease);
- Type V (McArdle's disease);
- Type VI (Hers' disease);
- Type VII (Tarui's disease);
- Type VIII disease.

Periodic acid-Schiff stain identifies glycogen and is useful in identifying these diseases.

GSD type II is an autosomal recessive metabolic disorder, caused by an accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucosidase enzyme (GAA). [3] It is also called Pompe disease or an acid maltase deficiency and is a rare multi-system hereditary storage disease that is progressive, and often fatal. [4] Nowadays, more than 350 gene mutations causing this disease have been identified and their number is constantly growing. [5]

The exact prevalence of Pompe disease is unknown. According to various authors, the incidence of the disease, depending on the country and ethnicity, ranges from 1:40,000 to 1:300,000. A 'founder effect' cannot be excluded. [6]

THE AIM

The purpose of this study is to provide a comprehensive narrative review of terminology, etiology, epidemiology, clinical manifestations, complications, and prognosis of Pompe disease, illustrated by a clinical case presentation of late-onset Pompe disease in a white child.

MATERIALS AND METHODS

A complex clinical, neurological, laboratory, and instrumental analysis provided to a child, admitted to the Regional Clinical Center of Neurosurgery and Neurology, Uzhorod city, Ukraine, resulted in diagnosis of GSD type II.

A comprehensive electronic literature search on Ovid, PubMed, Scopus, Embase, Cochrane, Google Scholar, and World Health Organization databases was performed to identify articles that discussed the neurological manifestations, presentations, complications, and prognosis of Pompe disease. The applicable articles are cited and referenced. No limit placed on publication time or the language of the article. All the relevant articles were identified and screened by two authors (HP and OF), and disagreements were resolved by consensus and involvement of senior authors (MO, YH, OS, YF); the results are summarized narratively.

CLINICAL CASE

We provided a complex clinical and instrumental analysis of manifestations and complications of Pompe disease in a white child, admitted to the Regional Clinical Center of Neurosurgery and Neurology, Uzhorod city, Ukraine. Clinical case presentation is accompanied with a comprehensive narrative review of terminology, etiology, epidemiology, clinical manifestations, complications, and prognosis of Pompe disease, presented in the discussion.

We report a case of a late-onset form of Pompe disease in a child resident of the Transcarpathian region.

Ten-year-old boy brought to his primary care physician by his parents because of difficulties in walking and climbing stairs, rapid fatigue, and shin pain after exercise during the last year. Examination revealed a positive Hoover›s test. Routine hematological tests were within normal limits. Total creatine kinase level was elevated to 1381 units. On the EMNG there were signs of pronounced diffuse myopathic syndrome; more severe in the proximal muscles.

TESTS

Taking into account the patient's complaints, a genetic scan for Duchenne muscular dystrophy was included in the diagnostic search plan but no deletions of the dystrophin gene exons were detected.

Pompe disease diagnostic was conducted. In the dried spots, low activity of GAA was detected, in connection with which molecular genetic diagnostics of GAA gene was assigned. A mutation was detected in the heterozygous state p.32-13T>G and c.307T>G. Signs of the vital organ involvement that is usual for Pompe disease were absent, including cardiomegaly or cardiomyopathy according to ECG and echocardiography.

GENEALOGICAL ANAMNESIS

A dry blood spot testing was performed for the father, mother, and sister. GAA activity was within normal range.

DIAGNOSIS AND TREATMENT

Thus, the patient was diagnosed with the metabolic disorder from the group of lysosomal accumulation diseases: Pompe disease (E 74.0).

The patient was referred to the Orphan Disease Center for the purpose of life-long enzyme replacement therapy (Miozim at a dose of 20 mg/kg intravenously once every 2 weeks).

Glycogen storage disease type II (GSD II) is a classical lysosomal storage disorder, characterized by lysosomal accumulation of glycogen and tissue damage, primarily in muscle and heart. [7] It has a broad continuous clinical spectrum in terms of onset, the involvement of organs, and life expectancy. In addition to muscle and heart involvement, other tissues affected are liver, spleen, endothelium, lung, brain, anterior horns, and peripheral nerves. The underlying enzyme deficiency is acid maltase (also known as GAA).

DISCUSSION

DEFINITION

GSD II is an autosomal-recessive disorder that results from the deficiency of GAA, a lysosomal hydrolase, and is part of a group of metabolic diseases called lysosomal storage disorders [3].

The disease was first described by the Danish pathologist Joannes Cassianus Pompe in 1932 when he was presented with a 7-month-old girl who died after developing idiopathic hypertrophic cardiomyopathy. Pompe observed an abnormal accumulation of glycogen in all examined postmortem tissues. He described the cardinal pathologic features of this lysosomal storage disorder.

ETIOLOGY

Pompe disease is caused by mutations in a gene that produces an enzyme called GAA. Absence or deficiency of GAA, a lysosomal enzyme that is responsible for the cleavage of the α-1,4- and α-1,6-glycosidic bonds of glycogen to glucose, leads to the accumulation of glycogen in the lysosomes in numerous tissues, but clinical symptoms are primarily due to cardiac and skeletal muscles involvement. [4]

CLASSIFICATION

Classification of GSD II is based on the age of onset, organ involvement, severity, and the rate of disease progression. There are three forms of GSD II.

- *Classic infantile* form refers to the form of Pompe disease that was first described in 1932 and characterized by the onset of symptoms shortly after birth: generalized muscle weakness, and cardiomegaly in combination with excessive glycogen storage in virtually all organs. [1]
- *Non-Classic infantile* form or so-called 'childhood', and 'juvenile' forms of Pompe disease are introduced as the names for the less severe forms of Pompe disease, characterized by delayed onset and usually slower progression.
- *Adult-Onset* or so-called 'late-onset' Pompe disease differs from infant form with milder clinical manifestations and course, absence of multiple organ pathology (heart damage is extremely rare), and more recent respiratory complications due to the weakness of the diaphragm and intercostal muscles.

EPIDEMIOLOGY

According to various authors, depending on the country and ethnicity, the incidence of GSD II is generally placed at approximately 5000 to 10000 births worldwide. [6] It occurs in various populations and ethnic groups around the world.

Approximately a third of GSD II patients are infants. Occurrence in the Netherlands is one in 138,000 infants. In China, Taiwan, and among African-Americans occurrence is one in 14,000. [6-8]

Table 1. Clinical Presentations of Glycogen storage disease type II

Types of Pompe Disease	Onset	Findings	Prognosis
Classic Infantile Onset	First three months after birth	Failure to thrive Lung infections Feeding problems Hearing problems Heart defects Hypertrophic cardiomyopathy Skeletal muscles weaknes Diaphragm and other breathing muscles weakness Enlarged liver Large tongue	Fatal within the first year of life
Non-Classic Infantile Onset	Later than the classic form but still appears within the child's first year of life	Failure to thrive Myopathy Lung Infections Feeding problems Hearing problems Abnormally enlarged heart Progressive muscle weakness Delayed development of motor skills	Poor and is often Fatal
Late-Onset	Any age	Myopathy Progressive diaphragm weakness Mobility problems High chance of falls Breathing problems Shortness of breath Frequent lung infections Morning headaches Tiredness Weight loss Difficulty swallowing Difficulty hearing Scoliosis, or a curved spine.	Poor and is often Fatal

The exact incidence of late-onset Pompe disease worldwide is unknown. A study in the Netherlands estimates that one in 57,000 adults has late-onset Pompe disease. [8] In Ukraine seven patients (1 adult, 6 children) were diagnosed with Pompe disease.

CLINICAL PRESENTATION

GSD II has a broad clinical spectrum. First symptoms can occur at any age from birth to late adulthood. Earlier onset compared to later onset is usually associated with faster progression and greater disease severity (Tabl. 1). At all ages, skeletal muscle weakness causes mobility problems and affects the respiratory system.

In table one we have narratively summarized the main clinical representations and outcomes of different forms of Pompe disease.

CLASSIC INFANTILE ONSET POMPE DISEASE

The infantile-onset form of the disease is the result of complete or near-complete deficiency of GAA. It is caused by mutations that lead to the production of less than 2 percent of functional GAA. [9, 10]

Symptoms begin in the first months of life and are characterized by a severe progressive course and rapid development of multiple organ pathology. Early symptoms include difficulty gaining weight and failure to grow at a normal rate, known as "failure to thrive." These infants experience feeding problems, poor weight gain, muscle weakness, breathing problems, lung infections, floppiness, and head lag. Many infants with Pompe disease also have enlarged liver and tongue.

Symptoms of malnutrition are due to the weakness of the facial muscles. Respiratory difficulties are due to the weakness of the diaphragm. Intercostal muscles are often complicated by lung infections. The heart is grossly enlarged due to hypertrophic cardiomyopathy that results in heart failure.

The condition is often fatal within the first year of life, but rapid treatment can reduce the risk of heart failure. Without timely treatment, most babies die from cardiac or respiratory complications before their first birthday. [11]

NON-CLASSIC INFANTILE-ONSET POMPE DISEASE

Non-classic infantile-onset Pompe disease begins later than the classic form but still appears within the child's first year. Despite the abnormally enlarged heart, the chance of

heart failure is lower compared to classic-infantile-onset form. Progressive muscle weakness leads to the delayed development of motor skills such as rolling over and sitting. Infants with this type of Pompe disease often experience severe respiratory problems due to damage and weakness in the muscles involved in breathing. [12]

The condition has a poor prognosis and is often fatal.

LATE-ONSET POMPE DISEASE

Late-onset Pompe disease is the result of a partial deficiency of GAA. The onset can be as early as the first decade of childhood or as late as the sixth decade of adulthood. [13, 14]

Late-onset Pompe disease is often milder and progresses more slowly than the infantile forms. In general, the later the disease appears, the slower the symptoms progress. It differs from infant form with milder clinical manifestations and course, absence of multiple organ pathology, and more recent respiratory complications due to the weakness of the diaphragm and intercostal muscles. [15] The primary symptom is muscle weakness progressing to respiratory weakness and death from respiratory failure after a course lasting several years.

Heart involvement is reduced in most cases of late-onset Pompe disease, but some patients may experience an irregular heartbeat or an enlarged heart. [16] However, as the disease progresses, breathing problems may increase and the most common cause of death is lung failure. Increased muscle weakness will often result in patients having to use mobility assistance, such as wheelchairs.

Late-onset Pompe disease patients usually die from respiratory failure and infectious pulmonary complications, depending on the time of onset and subsequent course of the disease. It may occur in childhood, adolescence, adulthood, or old age.

DIAGNOSTIC METHODS

A diagnosis of Pompe disease can be confirmed by screening for the common genetic mutations or measuring the level of GAA enzyme activity in a blood sample. [1, 17] Once Pompe disease is diagnosed, testing of all family members and a consultation with a professional geneticist are recommended. Carriers are most reliably identified via genetic mutation analysis.

CONCLUSIONS

Along with other lysosomal diseases, in the case of Pompe disease, it is possible to carry out pathogenetic enzyme replacement therapy which allows us to modify the course of the disease significantly, improve the quality of patients' lives and prevent the development of critical complications. The key to the successful use of enzyme replacement therapy is the early diagnosis of GSD II. A careful study of the target categories of patients suffering from myodystrophy and other myopathic syndromes at any age is required to identify late-onset Pompe disease so that pathogenetic treatment is started as early as possible.

We presented a comprehensive narrative review of the terminology, etiology, epidemiology, clinical manifestations, complications, and prognosis of Pompe disease, accompanied by a clinical case report of late-onset Pompe disease, to raise awareness about the GSD II.

REFERENCES

1. Roach P.J. Glycogen and its metabolism. Curr Mol Med. 2002;2:101–120. DOI: 10.2174/1566524024605761.
2. Saltik I.N., Ozen H., Ciliv G. et al. Glycogen storage disease type Ia: frequency and clinical course in Turkish children. Indian J Pediatr. 2000;67:497–501. DOI: 10.1007/BF02760476.
3. Kishnani P.S., Steiner R.D., Bali D. et al. Pompe disease diagnosis and management guideline. Genet Med 2006;8:267-288. doi: 10.1097/01.gim.0000218152.87434.f3.
4. American Association of Neuromuscular & Electrodiagnostic Medicine. Diagnostic criteria for late-onset (childhood and adult) Pompe disease. Muscle Nerve. 2009;40(1):149-60. doi: 10.1002/mus.21393.
5. Yang C.F., Liu H.C., Hsu T.R. et al. A large-scale nationwide newborn screening program for Pompe disease in Taiwan: Towards effective diagnosis and treatment. Am J Med Gen Part A 2014;164:54-61. DOI: 10.1002/ajmg.a.36197.
6. Vissing J., Lukacs Z., Straub V. Diagnosis of Pompe disease: muscle biopsy vs blood-based assays. JAMA neurology. 2013;70:923-927. doi: 10.1001/2013.jamaneurol.486.
7. Hirschhorn R., Reuser A. Glycogen storage disease type II: Acid alpha-glucosidase (acid maltase) deficiency. In: The metabolic and molecular bases of inherited disease. 2001: 3389-3419. doi: 10.1036/ommbid.417
8. Ausems M.G., Verbiest J., Hermans M.P. et al. Frequency of glycogen storage disease type II in The Netherlands: implications for diagnosis and genetic counselling. Eur J Hum Genet. 1999;7(6):713-6. doi:10.1038/sj.ejhg.5200367.
9. Winchester B., Bali D., Bodamer O.A. et al. Methods for a prompt and reliable laboratory diagnosis of Pompe disease: report from an international consensus meeting. Mol Genet Metab. 2008;93(3):275-81. doi: 10.1016/j.ymgme.2007.09.006.
10. Kroos M., Hoogeveen-Westerveld M., Michelakakis H. et al. Update of the Pompe disease mutation database with 60 novel GAA sequence variants and additional studies on the functional effect of 34 previously reported variants. Human mutation 2012;33:1161-1165. doi: 10.1002/humu.22108.
11. Chien Y.H., Lee N.C., Chen C.A. et al. Long-term prognosis of patients with infantile-onset Pompe disease diagnosed by newborn screening and treated since birth. The Journal of pediatrics 2015;166(4):985-991. DOI: 10.1016/j.jpeds.2014.10.068.
12. Bay L.B., Denzler I., Durand C. et al. Infantile-onset Pompe disease: Diagnosis and management. Arch Argent Pediatr. 2019;117(4):271-278. doi: 10.5546/aap.2019.eng.271.
13. Kishnani P.S., Howell R.R. Pompe disease in infants and children. J Pediatr. 2004;144(5 Suppl):S35–S43. DOI: 10.1016/j.jpeds.2004.01.053.
14. Burton B.K. Newborn screening for Pompe disease: An update, 2011. Am J Med Genet C Semin Med Genet. 2012;160C:8–12. doi: 10.1002/ajmg.c.31315.
15. Müller-Felber,W., Horvath R., Gempel K. et al. Late onset Pompe disease: clinical and neurophysiological spectrum of 38 patients including long-term follow-up in 18 patients. Neuromuscular Disorders. 2007;17(9-10):698-706. DOI: 10.1016/j.nmd.2007.06.002.
16. Chan J., Desai A.K., Kazi Z.B. et al. The emerging phenotype of late-onset Pompe disease: A systematic literature review. Molecular genetics and metabolism. 2017;120(3):163-172. doi: 10.1016/j.ymgme.2016.12.004.

17. Musumeci O., la Marca G., Spada M. et al. LOPED study: looking for an early diagnosis in a late-onset Pompe disease high-risk population. Journal of Neurology, Neurosurgery & Psychiatry. 2016;87(1):5-11. doi: 10.1136/jnnp-2014-310164.

ORCID and contributionship:
Hanna V. Palahuta: 0000-0001-7348-4390 [A,B,D,E,F]
Olena Y. Fartushna: 0000-0002-4641-0836 [A,B,D,E,F]
Olha G. Selina: 0000-0002-8291-5583 [E,F]
Yevhen M. Fartushnyi: 0000-0002-5199-5373 [E,F]
Tetiana V. Koval: 0000-0001-7256-7901 [E]

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CORRESPONDING AUTHOR
Olena Y. Fartushna
Ukrainian Military Medical Academy
24 Melnikova St., 04050 Kyiv, Ukraine
tel: +38 094 847 04 56
e-mail: olena.y.fartushna@gmail.com

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CASE STUDY

TOTAL MANDIBULAR DEFECT RECONSTRUCTION BY TOTAL TITANIUM PATIENT-SPECIFIC IMPLANT: CLINICAL EFFICACY AND LONG TERM FOLLOW UP. CLINICAL CASE

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Denis M. Chernohorskyi, Yuriy V. Chepurnyi, Oleksandr A. Kanyura, Andriy V. Kopchak
[1]O.O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE
[2]KYIV REGIONAL CLINICAL HOSPITAL, KYIV, UKRAINE

ABSTRACT

The aim of this clinical case in demonstrating the possibility of replacing total defect of the mandible with a patient specific implant and the result of long-term follow up.

Literature data on the replacement of total mandibular defects are extremely limited and they are presented by only several clinical cases where various surgical approaches were used. In the available literature, there are two approaches to solving this problem, including the replacement of the jaw with vascularised bone grafts, of which the fibula flap is the most promising, and the implantation of endoprostheses of the jaws, of which patient-specific anatomical endoprostheses made using additive technologies are the most advanced. The concept of using patient-specific endoprostheses of the whole mandible is considered revolutionary because it has a number of significant benefits, including the greatest accuracy in restoring the anatomical shape of the mandible.

One of the unresolved problems associated with the installation of total mandibular endoprostheses is the prosthetic rehabilitation of patients using fixed structures. The analysis of the presented case can be a good tool for the clinician and bioengineer while making the final decision on the treatment method and modality in patients who need an identical option for the repair of a mandibular defect.

Based on CT data, we can conclude that the employed approach, methodology of design and manufacture of patient-specific titanium mandibular endoprosthesis for the total defect demonstrated the sufficient efficacy, which suggest the need for further systematic studies to address this issue.

KEY WORDS: Patient specific implant, total mandibular defect, mandible segmentation, mandible reconstruction

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INTRODUCTION

Total or subtotal mandibular defects are one of the most pressing and challenging issues in modern maxillofacial surgery. This is due to the fact that the loss of the lower jaw, or a significant part of it inevitably results in profound functional disorders (difficulty chewing, swallowing, breathing and speaking), as well as severe cosmetic deficiencies, facial distortion, social maladaptation, permanent disability, mental disorders and decreased quality of life [1]. The main causes of total and subtotal mandibular defects include tumours [2], osteomyelitis [3], radiation and drug-associated osteonecrosis, congenital malformations such as hemifacial microsomia, the first and second branchial arch syndrome, Pierre Robin sequence and Goldenhar syndrome [4].

In recent decades, a number of approaches have been offered to treat large mandibular defects, which includes those based on the use of 1) free bone grafting procedures [5] or free tissue transferring [6]; 2) conventional reconstructive plates or endoprostheses, based on computer-aided design and manufacturing (CAD/CAM); 3) the combination depending on the clinical situation [7]. Whatever the case, to achieve optimal anatomical and functional outcomes, the authors recommend the use of virtual simulation of surgery and customisation of implants and fixators (plates, grids, etc.) through model-based bending, milling or additive technologies (DSLM) [8].

Until recently, surgeons most widely used microvascular fragmented fibula transferring, usually without reconstruction of the TMJ, forming pseudoarthrosis at the ends of the bone graft [9]. This technique demonstrated acceptable aesthetic and functional outcomes owing to the possibility of dental implants placement and appropriate prosthetic reabilitation. On the other hand, the authors point to a number of problems and limitations associated with microvascular bone grafting in the area of total mandibular defects, such as a sharp deterioration in the blood supply to the lower third of the face, or traditional recipient vessels may be compromised or absent. Especially, this could be typical defects, caused by drug-associated or radiated osteonecrosis [10].

Numerous prospective and retrospective studies have proven the efficacy of CAD/CAM technology and microvascular tissue transferring for the replacement of mandibular defects [11, 12, 13, 14]. However, the mandibular reconstruction is much more challenging in patients with total mandibular defects [15]. The number of publications concerning total defects is extremely limited, and all of them have data on individual clinical cases. From a topo-

graphic, anatomical and technical point of view, total jaw defects are extremely tough to replace. Due to the very small number of well-documented cases, it is extremely difficult to formulate clear guidelines for the treatment of patients with total mandibular defects. [12]

In 2009, the Dutch company Xilloc together with LayerWise offered a new concept for the treatment of total mandibular defects and an original design for the solid titanium endoprosthesis manufacturing. The developed design was successfully implanted by Belgian professor Jules Poukens in a patient who had needed the lower jaw removal due to bisphosphonate osteonecrosis. In 2011, Professor Jules Poukens and Xilloc CEO Maikel Beerens reported the successful installation of a complete mandibular endoprosthesis in an 83-year-old patient with progressive osteomyelitis [16].

The benefits of this approach include the maximum compliance with the anatomical shape of the patient's lower jaw and an alternative to free tissue transferring in complex cases. At the same time, there is almost no data in the literature on the efficacy of the technique, possible risks and behaviour of the structure with long-term follow up.

Below, we present the clinical case demonstrating our experience of using a total mandibular endoprosthesis in a patient with the total defect, and assessment of the immediate and long-term (more than 2 years) postoperative outcomes.

CASE REPORT

Aim of this clinical case in demonstrating the possibility of replacing total defect of the mandible with a patient specific implant and the result of long-term follow up.

All performed procedures were in accordance with the ethical standards of the institutional and national research committee, and with the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. The study was approved by the Bogomolets National Medical University Bioethics Committee (Protocol No 126). Writhen informed consent were obtained from all persones included in the study.

Patient K., 27 years old female, presented to the Centre of Maxillofacial Surgery and Dentistry, Kyiv Regional Clinical Hospital with complaints about an aesthetic defect (sharp deformation of the lower third of the face and upper neck, lower lip, complete secondary adentia) and severe functional deficiency (inability to chew food, difficulty speaking and swallowing, salivation, inability to fully close the lips, sleep apnoea) due to a total mandibular defect. The patient considered the defect to be the cause of deep psycho-emotional disorders (depression, neurasthenia) and a sharp reduction in the social contacts.

The etiology of the defect was associated with the destruction and the mandibular sequestration due to a prolonged diffuse infectious and osteonecrosis due to the systematic use of artisanal derivatives of pervitin [17]. The patient had had this condition for more than 11 years and she underwent 10 surgical interventions (sequestrectomy) with ligation of both external carotid arteries. A long-lasting inflammatory process and previous surgical interventions resulted in marked scarring and atrophy of the soft tissues of the mouth floor and upper neck.

Before starting treatment, the patient had not taken narcotic agents for more than 5 years. The application of the traditional approach with the fibula flap transferring in this case was challenging due to the following factors: the lack of vessels, adequate for anastomosis in the recipient site, the systemic damage to the endothelial lining of vessels caused by pervitin, and the patient's concern about possible complications in the donor site. We took a decision to apply for reconstruction a patient-specific total mandibular endoprosthesis, based on a design offered by Xilloc in 2009.

Before surgery she was evaluated with contrast CT scans and angiography of the head and neck region, as well as both legs. The mandible reconstruction was preceding by an expander placement to create the space for future total patient-specific mandibular endoprosthesis. The expander was installed into anterior part of the floor of the mouth for a period of 4 weeks.

DESIGN AND MANUFACTURE OF TOTAL MANDIBULAR ENDOPROSTHESIS

CT scans of the patient, as DICOM files (Digital Imaging and Communications in Medicine) were imported into the software environment D2P (DICOM-2-PRINT former Simbionix) ver.1.0.253, 3D Systems, Israel), were CT segmentation was performed with following virtual 3D models' calculation of the facial skull and surrounding soft tissues. To select the parametrically optimal mandible, which was to act as a prototype of the future complete mandibular endoprosthesis, the anthropometrically corresponding mandible was imported, as virtual model following the same segmentation procedures from the CT study of another anonymous patient.

The models, which was created in D2P (3D Systems, USA) as a STL file, were exported to the Geomagic Freeform Plus (version 2016.0 Hotfix 2), where fixing of the model geometry errors was performed. Next, the optimal size and shape of the mandible were determined using the Alignment function and multi-stage verification (profile line, partial cephalometric analysis, soft tissue reproduction, etc.). The "donor" jaw of a certain size was modified in the area of the mandibular rami, creating holes that lightened the construction and served as retention points for attaching the masticatory muscles. Coronal processes were removed from the model, and smaller holes were added along the base of the jaw. The frontal part of the jaw was flattened and rounded, and in the lower third fixation holes were made for a group of muscles of the mouth floor. At the top of the alveolar part of the future implant, there were simulated four fixation holes for fixing the supports of the future prosthetic structure. The volume and linear size of the condyles were also reduced. The three-dimensional model of the created design is shown in Fig. 1.

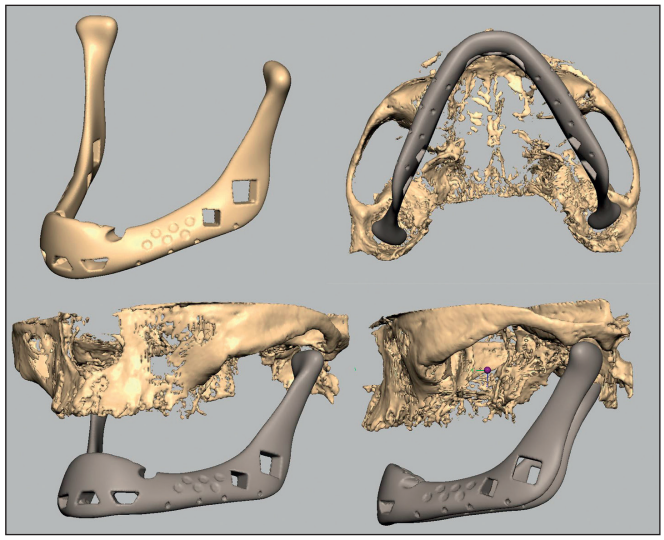

Fig. 1. Virtual design of the full mandible patient specific implant

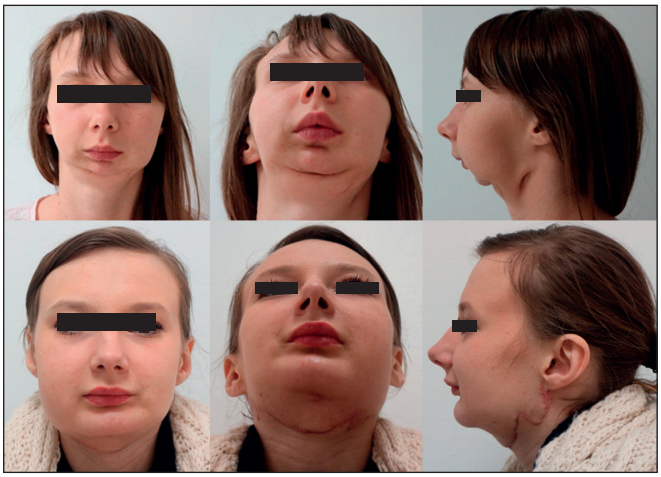

Fig. 2. Patient photos with whole mandible defect: before (upper) and after operation (lower).

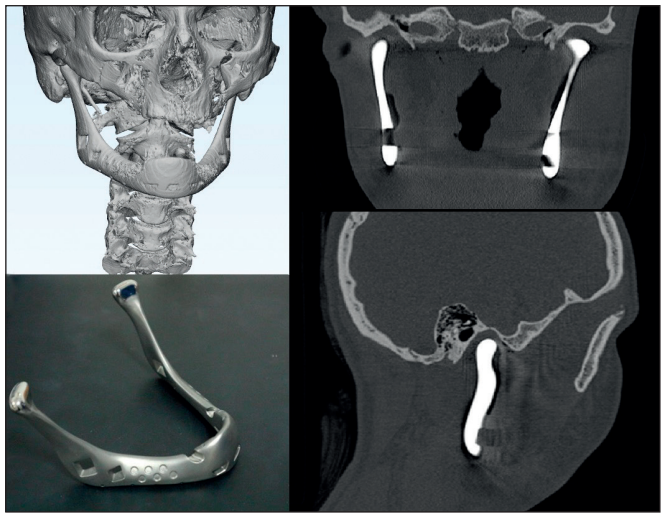

Fig. 3. Full mandible patient specific implant and CT 3D reconstruction with CT images slices 1 month after surgery.

MANUFACTURE OF IMPLANTS USING ADDITIVE TECHNOLOGIES

The titanium endoprosthesis was made of Grade 5 (Ti6Al4V) medical titanium, using DMLS technology by European 3D Systems Printing Centre (former Layerwise) Leuven, Belgium. After "printing", the area of the condyle heads was ground to the mirror surface, while the change in metal thickness did not exceed 30 microns.

CLINICAL STAGE

Surgery was performed under general anesthesia through the extraoral approaches using previous scars: bilateral submandibular and submental, with preservation of the little skin islands between them. Total endoprothesis was installed under platysma into created space, the condyle heads of the implant were placed into the articular fossae, the chin was positioned in the midline, masseters, muscles of the thongue and suprahyoid grope were fixed to the endoprothesis. The patient was prescribed a course of antibacterial therapy and non-steroid analgesics. No complications were seen in the early postoperative period, except some limited mouth opening and foreign body sensation, she required the use of a nasogastric tube for adequate nutrition. The patient assesses the achieved aesthetic outcome as good (Fig. 2).

She refused to have a fixed prosthetic structure installed due to the risk of infection and loss of the structure. Therefore, 4 months following the operation, the patient was installed a removable prosthesis with a soft base. During the entire observation period (27 months), no infectious complications, exposure of the structure or the displacement from the initial position were noted. (Fig. 3)

Literature data on the replacement of total mandibular defects are extremely limited and they are presented by only several clinical cases where various surgical approaches were used. There are currently no clear, scientifically sound guidelines for the replacement of such defects, or the comparison of the clinical efficacy of different techniques. Concurrently, much experience has been gained in the treatment of segmental and subtotal mandibular defects because the availability of preserved parts of the jaw, joint elements and muscle fixation points makes it possible to more accurately determine the desired anatomical shape and position of the lower jaw, as well as more fully restore its functional parameters (the nature of movement in joint, chewing stereotype, etc.) [18]. Therefore, the publication of well-documented case reports of treatment of total mandibular defects with a long observation period and their comparison with previously published data is of considerable interest.

In the available literature, there are two approaches to solving this problem, including the replacement of the jaw with vascularised bone grafts, of which the fibula flap is the most promising, and the implantation of endoprostheses of the jaws, of which patient-specific anatomical endoprostheses made using additive technologies are the

most advanced [19]. The use of the fibula to replace total defects is promising for the following reasons: 1) this method allows restoring the mandible owing to the vital bone tissue that is resistant to infection and integrates well with surrounding soft tissues; 2) the possibility of performing a number of osteotomies allows restoring the mandibular anatomy with high accuracy, as evidenced by satisfactory aesthetic results reported by the authors; 3) the successful installation of dental implants in the site of transplanted fibula demonstrated by van Baar et al. (2018) allowed effective restoring the masticatory function with high-quality prosthetic structures [20]; 4) the possibility of bone grafting together with soft tissues, which can simultaneously replace complex combined defects, 5) the experience of widespread use of this flap in the replacement of smaller defects makes the transplant technique quite predictable in terms of immediate and long-term results. However, the technique has obvious shortcomings and limitations related to the condition of hemodynamics and soft tissues of the donor and recipient sites, systemic diseases of bone tissue and metabolic disorders, risks of flap harvesting, which often cause the patient to refuse surgery and the like.

These problems are most pronounced in the group of patients with drug-associated, toxic and radiation osteoneonecrosis, which is the most common cause of total mandibular defects. The presented case demonstrates that the transplantation of a free fibula flap was rather challenging and the risk of such an intervention was very significant.

The concept of using patient-specific endoprostheses of the whole mandible is considered revolutionary because it has a number of significant benefits, including the greatest accuracy in restoring the anatomical shape of the mandible, no need for additional trauma to the donor site, a significant reduction in operative time and duration of postoperative rehabilitation, no restrictions associated with the pathology of the great vessels.

The design of the structure in this study broadly reproduced the design offered by Xilloc, but the individual characteristics of the clinical case were taken into account. After the "donor" idealized mandibular model had been imported and cephalometric analysis had been performed, the most optimal size and shape of the mandible were determined. At the next stage, a patient-specific implant was shaped using sequential algorithmic transformations of the prototype with software design tools. Particular attention was paid to the reduced structure weight without loss of the strength and integration of the endoprosthesis with soft tissues.

The proper implant placement, which was radiologically confirmed, improved the aesthetics of the face in the early postoperative period and ensured a significant recovery of lost functions. Concurrently, such an approach raises concerns about the behaviour of the structure during the long-term operation under chewing load. The absence of infectious complications or exposure of the structure in our observation is suggestive of the potential possibility of using such endoprostheses not only as a temporary solution preceding the final bone grafting, but also as a long-lasing resolution, including lifelong. It should be noted that although the experience of using patient-specific endoprostheses shows a low incidence of complications following the repair of smaller mandibular defects, the incidence of complications in the replacement of total defects may be higher [21].

One of the unresolved problems associated with the installation of total mandibular endoprostheses is the prosthetic rehabilitation of patients using fixed structures. The potential connection of the endoprosthesis with the oral cavity along the transmucosal elements increases the risk of the prosthetic structure loss. In our observation, the patient's refusal of a fixed structure does not allow us to draw any conclusions in this regard. However, our experience shows that the use of removable dentures prostheses is not a functionally complete resolution.

Another controversial issue is the feasibility of two-component patient-specific joint endoprostheses for the replacement of large defects of the jaws with the loss of the mandibular head. Some authors cover this issue [22]. However, they do not reach a consensus, and the results appear to be controversial. The approach employed in our case (with the existing loss of articular discs and components of the joint capsule) is similar to the solution offered by Imad Abu el-Naaj, and it did not provide for the complete restoration of the joint elements. Despite this, the adaptive self-adjusting mechanisms of the masticatory system made it possible to restore the basic parameters of mandibular movements simulating physiological opening and closing of the mouth, as there were no lateral movements (laterotrusive); the inability to contract masticatory muscles and chewing with considerable effort possibly reduced the pressure of the titanium heads on the bone elements of the articular fossa, which was confirmed by the absence of pain and bone remodeling in the site of contact with the endoprosthesis [23].

CONCLUSIONS

Overall, based on CT data, we can conclude that the employed approach, methodology of design and manufacture of patient-specific titanium mandibular endoprosthesis for the total defect demonstrated the sufficient efficacy, which suggest the need for further systematic studies to address this issue. In such a case, given the lack of CT data of the patient's jaws before the defect, the design of a total mandibular endoprosthesis is a complex bioengineering task that requires special methods and techniques, and the use of total endoprosthesis as such has its drawbacks and advantages. In addition, being very rare, this kind of work requires the involvement of expert-level bioengineers, which brings it closer to the level of exclusive. Therefore, the analysis of the presented case can be a good tool for the clinician and bioengineer while making the final decision on the treatment method and modality in patients who need an identical option for the repair of a mandibular defect.

REFERENCES

1. Al-Ahmari A., Abouel Nasr E., Moiduddin K. et al. A comparative study on the customized design of mandibular reconstruction plates using finite element method. Mechanical Engineering. 2015; 28(1):28-35.
2. Prein J., Hammer B. Reconstruction of extensive anterior defects of the mandible. Craniomaxillofacial Reconstructive and Corrective Bone Surgery. Springer, New York, NY. 2002.
3. Neville B.W., Day T.A. Oral cancer and precancerous lesions. CA Cancer J Clin. 2002;52(4):195-215.
4. Ashokan C.S., Sreenivasan A., Saraswathy G.K. Et al. Goldenhar syndrome – review with case series. J Clin Diagn Res. 2014;8(4):17-9.
5. Foster R.D., Anthony J.P., Sharma A.P. et al. Vascularized bone flaps versus nonvascularized bone grafts for mandibular reconstruction: an outcome analysis of primary bony union and endosseous implant success. Head Neck. 1999;21(1):66-71.
6. Schliephake H., Schmelzeisen R., Samii M. et. al. Microvascular reconstruction of the skull base: indications and procedures. J Oral Maxillofac Surg. 1999;57(3):233-9.
7. Wilde F., Hanken H., Probst F. et al. Multicenter study on the use of patient-specific CAD/CAM reconstruction plates for mandibular reconstruction. Comput Assist Radiol Surg. 2015;10(12):2035-51.
8. Duda T. 3D Metal Printing Technology. IFAC-PapersOnLine. 2016;49(29):103-110. DOI: 10.1016/j.ifacol.2016.11.111.
9. Disa J.J., Cordeiro P.G. Mandible reconstruction with microvascular surgery. Semin Surg Oncol. 2000;19(3):226-34.
10. Migliorati C.A., Schubert M.M., Peterson D.E. et al. Bisphosphonate-associated osteonecrosis of mandibular and maxillary bone: an emerging oral complication of supportive cancer therapy. Cancer. 2005;104(1):83-93.
11. Pogrel M.A., Podlesh S., Anthony J.P. et al.. A comparison of vascularized and nonvascularized bone grafts for reconstruction of mandibular continuity defects. J Oral Maxillofac Surg. 1997;55(11):1200-6.
12. Jeremic J.V., Nikolic Z.S., Boricic I.V. et al. Total mandibular reconstruction after resection of rare «honeycomb-like» ameloblastic carcinoma--a case report. J Craniomaxillofac Surg. 2010;38(6):465-8.
13. Okada Y., Mataga I., Katagiri M. et. al. An analysis of cervical lymph nodes metastasis in oral squamous cell carcinoma. Relationship between grade of histopathological malignancy and lymph nodes metastasis. J Oral Maxillofac Surg. 2003;32(3):284-8.
14. Matros E., Santamaria E., Cordeiro P.G. Standardized templates for shaping the fibula free flap in mandible reconstruction. J Reconstr Microsurg. 2013;29(9):619-22.
15. Sato J., Yamazaki Y., Satoh A. et al. Pain may predict poor prognosis in patients with oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;111(5):587-92.
16. Nickels L. World's first patient specific jaw implant. Elsevier Metal Powder Report. 2002; 67(2):12–14.
17. Poghosyan Y.M., Hakobyan K.A., Poghosyan A.Y. et al. Surgical treatment of jaw osteonecrosis in «Krokodil» drug addicted patients. J Craniomaxillofac Surg. 2014;42(8):1639-43.
18. Sirak S.V., Sletov A.A., Shchetinin E.V., et al. Treatment and rehabilitation of patients with subtotal mandibule defects. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2015: 1803–1810.
19. Pellini R., Mercante G., Spriano G. Step-by-step mandibular reconstruction with free fibula flap modelling. Acta Otorhinolaryngol Ital. 2012;32(6):405-9.
20. van Baar G.J.C., Forouzanfar T., Liberton N.P.T.J. et al. Accuracy of computer-assisted surgery in mandibular reconstruction: A systematic review. Oral Oncol. 2018;84:52-60.
21. Mazzoni S., Marchetti C., Sgarzani R. et al. Prosthetically guided maxillofacial surgery: evaluation of the accuracy of a surgical guide and custom-made bone plate in oncology patients after mandibular reconstruction. Plast Reconstr Surg. 2013;131(6):1376-85.
22. Wolford L.M., Mercuri L.G., Schneiderman E.D. et al. Twenty-year follow-up study on a patient-fitted temporomandibular joint prosthesis: the Techmedica/TMJ Concepts device. J Oral Maxillofac Surg. 2015;73(5):952-60.
23. Wolford L.M., Mehra P. Custom-made total joint prostheses for temporomandibular joint reconstruction. Proc (Bayl Univ Med Cent). 2000;13(2):135-8.

The research approved by Bioethics Committee of Bogomolets National Medical University, Kyiv, Ukraine (Protocol No. 107).

ORCID and contributionship:

Denis M. Chernohorskyi: 0000-0002-1618-5930 [A,B,D,E]
Yuriy V. Chepurnyi: 0000-0003-4393-3938 [A,E,F]
Andriy V. Kopchak : 0000-0002-3272-4658 [A,E,F]
Oleksandr A. Kanyura 0000-0002-6926-6283 [A,E,F]

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Denis M. Chernohorskyi
Bogomolets National Medical University
13 T. Shevchenko blvd, 01601 Kyiv, Ukraine
tel:+380990889397
e-mail: cher103@meta.ua

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CASE STUDY

A RARE COMPLICATION OF CALCANEAL FRACTURE – CALCANEAL NON-UNION. CASE REPORT

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Andrii A. Sherehii, Vasil V. Stoika, Vasil V. Lytvak
SHEI "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE

ABSTRACT

The aim was to study published reports of postoperative intra-articular calcaneus fractures non-union complications after ORIF, determinate the main reasons for such complications and study the ways to improve the treatment results/ We retrospectively studied anamnestic data, medical history from 2018, and the treatment process of our patient in 2019-2020 with calcaneus ORIF post-operative non-union. The patient was managed by secondary ORIF with autogenous bone marrow grafting after removal of broken retainers. At 6 months, the result was evaluated as good according to the AOFAS scale, (89–80 points for AOFAS). Non-union after calcaneus fracture ORIF management is an extremely rare complication. Controlled randomized trials with a larger sample size and longer follow-up are required for plausible evidence and systematization of treatment principles.

KEY WORDS: clinical case, calcaneus fracture, non-union

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INTRODUCTION

The most vulnerable to calcaneal fractures are physically active, able-bodied male. In the observation of Savgachev, 2016, which was based on 372 patients aged 22 to 58 years with a peak of 42 ± 3 years, for the period 2010 - 2016, gender distribution was more favorable for male patients (81.6%) than for female patients – 69 (18.4%) [1].

According to the statistics given by Essex-Lopresti P., calcaneus fractures in 75% -92% of cases have a fracture line that runs through the articular surface and are characterized as intraarticular with an impression-shifting nature. It can be a prerequisite for post-traumatic deforming osteoarthrosis of the joints of the posterior foot, with the consequences in the form of constant pain, swelling syndromes, functional restriction of the foot [2].

Relatively infrequent, but at the same time severe in consequence, is trauma of the posterior part of the foot, including fracture of the calcaneus, which makes from 2,0% to 5,7% in the structure of all fractures of the human skeleton, and taking up nearly 65% of the total foot bones fractures [3].

The high rate of unsatisfactory treatment results in the range of 13.0% –27.3% is maintained nowadays, and in some types of fractures their proportion reaches up to 80.5% [4]. The need for re-surgical treatment arises in 40% of such patients in 2–3 years after the trauma and the proportion of entry to primary disability as a whole range from 25.0% to 34.1% of all victims [5]. However, calcaneal non-union after surgical treatment is a rare complication, which was described only in severe case reports available for our review.

The basis of modern principles of treatment is the restoration requirement of joint congruence, restoration of axial ratios under the condition of careful attitude to soft tissues.

Our research intended to study published reports of postoperative intra-articular calcaneus fractures non-union complications after ORIF, determinate the main reasons for such complications and study the ways to improve the treatment results and identify the best clinical treatment strategies for their management.

We retrospectively studied anamnestic data, medical history from 2018, and the treatment process of our patient in 2019-2020 with calcaneus ORIF post-operative non-union at Trauma department of Uzhhorod district clinical hospital of Uzhhorod district council of Zakarpattia region.

Assessments of the clinical efficiency of surgical treatment complexes were studied in terms of 3 months, and 6 months using X-ray and clinical-functional methods.

Literature retrieval composed by the searched publication through databases PubMed, Medline, Elsevier, Google scholar and E-library using the search terms "calcaneal fracture complications", "Heel-bone malunion", "calcaneus non-union". The search was restricted to English-language and Russian-language journals.

CASE REPORT

A 56 years old male patient was referred to the Uzhhorod district clinical hospital by family GP doctor with persistent pain and swelling of his heel during physical activity, walking distance over 400 meters.

ANAMNESIS MORBI

The patient received injury, as a result of falling from a height in 2018. He accepted emergency care in the amount of plaster

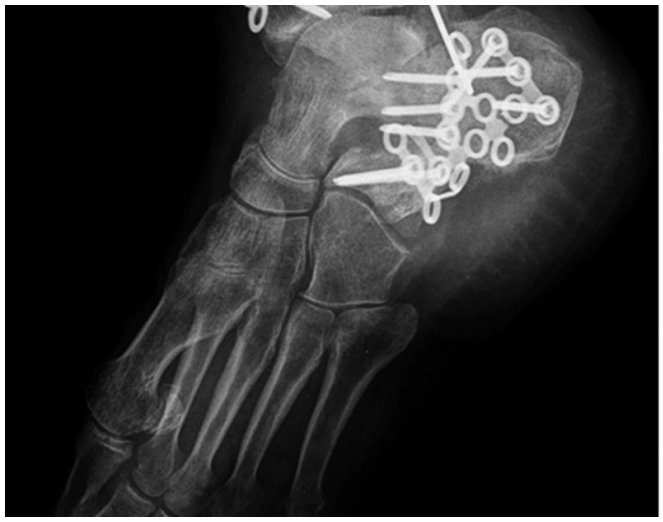

Fig. 1. Radiographs 5 months after the accident.

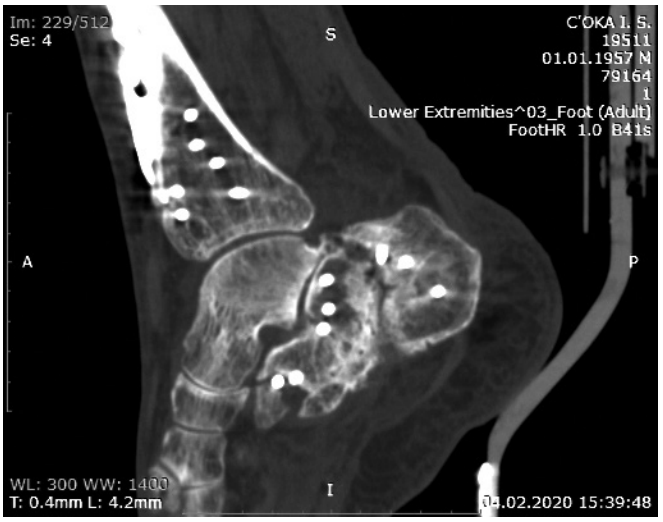

Fig. 2. CT- print 5 months after the accident.

immobilization, analgesics. Patient's general condition was burdened by the presence of edema, epidermal phlyctens, subcutaneous hematomas of the foot and ankle joint, over 20 years smoking experience. Next day after the trauma he was admitted to the trauma department of the City Hospital with Diagnosis: Closed calcaneus intraarticular AO 82 C2 (Sanders 3) fracture, with joint depression and fragment displacement. The conservative treatment for twelve days by anti-edema therapy, applying plaster cast immobilization, physical therapy by isometric muscle exercises of the thigh and shin from both lower extremities led soft tissues to the readiness for surgery. Applied ORIF with restoration of the posterior talar articular surface of the calcaneus and LCP fixation, followed by protected weight bearing for 9 weeks. Postoperative period without complications during observation period.

THE MEDICAL HISTORY (ANAMNESIS VITAE)

The working age patient was in good physical condition, had any significant comorbidities before the trauma accident. For the last 3 years suffered from a periodic hypertension with values of systolic blood pressure up to 150 mm. Hg., cured by occasional intake of oral medications, composed of Amlodipini besilas. Smoker's experience is occurring more than last 10 years. The osteochondrosis of the lumbosacral region of the spine have been troubling for 5-6 years.

The patient denies the presence of viral hepatitis, tuberculosis, HIV, venereal diseases in the medical history; allergic history is not burdened

PHYSICAL EXAMINATION (STATUS PRAESENS OBJECTIVUS)

Clinical examination and radiological evaluation, including CT scan of the foot, revealed evidence of calcaneal non-union with subtalar arthritis and broken plate in February 2020 (Fig. 1., Fig. 2.). During pre-operative assessment any serious comorbidity wasn't detected. \

TREATMENT

The patient was managed by secondary ORIF with autogenous bone marrow grafting after removal of broken retainers. Ultimate stabilization was achieved with a heel plate with angular stability and locking screws. In the early postoperative period superficial surgical site infection combined with marginal superficial 2 cm necrosis of the proximal skin flap was developed, that has been successfully treated with regular wound dressings and antibiotics. Delayed wound healing allowed to remove the stitches in 15 days and did not significantly affect the final result of treatment.

At 6 months, the result was evaluated as good according to the AOFAS scale, (89–80 points for AOFAS). On standard radiographs, depression of the Böhler angle was noted within 8°-10° degrees.

We have received only a few case reports from accessible sources on English and only single review on Russian language. The first reported case of NON-UNION after calcaneal fracture was published by Thomas in 1993 as recent [6]. There is no significant gender difference in incidence between male or female even in the small number of cases reported. Due to the small number of cases it was not possible to identify similarities that could indicate as a risk factor for non-union. According to the data published by Zwipp et al. there is a tendency to Non-Union preferably after conservative treatment in theirs experience with high rank of 10% [7]. Smoking and Diabetes mellitus has been detected as a probable risk factor. In Karakurt et al. Report all patients were smokers [8]. This assumption is confirmed by our case with long term smoking experience in patient.

Wajdi's study substantiates the possibility of using Bone Marrow Concentrates (BMC) or Platelet Rich Plasma (PRP) methods, with satisfactory results without harming the subtalar joint [9]. Calcaneal Non-Union refers to a rare but serious complication after calcaneal fractures. The weakness of Zhangs and other studyes is that case data are limited, and no more reliable conclusions can be drawn. Considerable high-quality, evidence-based studies are required to explore the etiology of the disease and appropriate treatment options in the future [10].

CONCLUSIONS

Non-union after calcaneus fracture ORIF management is an extremely rare complication.

Controlled randomized trials with a larger sample size and longer follow-up are required for plausible evidence and systematization of treatment principles.

REFERENCES

1. Savgachev V.V. Rangovaya model` riska razvitiya oslozhnenij pri travme pyatochnoj kosti [Rank Model of Complications Risk in Heel Trauma]. Postgraduate Doctor. 2016; 75(2):16-23. (In Russian).
2. Essex-Lopresti P., Peltier L.F. The mechanism, reduction technique, and results in fractures of the os calcis. Clinical Orthopaedics and Related Research. 1993;290:3-16.
3. Kumar N. Non-union of calcaneum – A rare complication of calcaneal fracture – A case report with brief review of literature. J Clin Orthop Trauma. 2015; 6(3): 187-189.
4. Assous M., Bhamra M.S. Should Os calcis fractures in smokers be fixed? A review of 40 patients. Injury. 2001; 32: 631-632.
5. Gehr J., Schmidt A., Friedl W. Calcaneus pseudarthrosis: a clinical rarity. Unfallchirurg. 2000;103(6): 499-503.
6. Thomas P., Wilson L.F. Non-union of an os calcis fracture. Injury. 1993; 24(9): 630-632.
7. Zwipp H., Tscherne H., Thermann H., Weber T. Osteosynthesis of displaced intraarticular fractures of the calcaneus results in 123 cases. Clinical Orthopaedics and Related Research®. 1993;290: 76-86.
8. Karakurt L., Yilmaz E., Belhan O. et al. Pseudarthrosis of a calcaneus fracture: a case report. Acta Orthop Traumatol Turc. 2004; 38(4): 288-290.
9. Wajdi B., Rebai M.A., Baya W. et al. Pseudarthrosis of the Calcaneus: Advantages of Regenerative Medicine in the Management of a Rare Entity. A Case Report and Review of Literature. Open Orthop J. 2018; 12: 141-146.
10. Zhang H., Cheng Yu., Cheng K., Hongtao H. Case Report Etiology and treatment of calcaneal NON-UNION: a report of two cases . Int J Clin Exp Med. 2020; 13(2): 1316-1322.

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ORCID and contributionship:

Andrii A. Sherehii: 0000-0002-0088-5473 [A,B,C]
Vasil V. Stoika: 0000-0003-2219-2544 [D,E]
Vasil V. Litvak: 0000-0002-3378-9648 [C,F]

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CORRESPONDING AUTHOR

Andrii A. Sherehii
SHEI "Uzhhorod National University"
3 Narodna sq. 88000, Uzhhgorod, Ukraine
tel: +38 (050) 610 09 76
e-mail: a.a.s_07@mail.ru

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CASE STUDY

ACUTE TRANSVERSE MYELITIS AS A NEUROLOGICAL COMPLICATION OF COVID-19: A CASE REPORT

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Hanna V. Palahuta[1], Olena Y. Fartushna[2], Stanislav K. Yevtushenko[3], Yana Y. Hnepa[4]

[1] STATE UNIVERSITY "UZHHOROD NATIONAL UNIVERSITY", REGIONAL CLINICAL CENTER OF NEUROSURGERY AND NEUROLOGY, UZHHOROD, UKRAINE

[2] UKRAINIAN MILITARY MEDICAL ACADEMY, KYIV, UKRAINE

[3] KHARKIV MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KHARKIV, UKRAINE

[4] STATE UNIVERSITY "UZHHOROD NATIONAL UNIVERSITY", UZHHOROD, UKRAINE

ABSTRACT

We aim to report a COVID-19-related case of acute myelitis that has not been associated with any other viral infections. A 23-year-old student was admitted to the hospital within a month from the time of loss of smell and taste with features of acute-onset non-compressive myelitis with paresthesia on both sides from the Th9 level. Complex neurological, clinical, laboratory, and neuroimaging examination was performed within 24 hours of admission. MRI of the spine showed a segment of increased T2 signal in the center of the spinal cord at Th11-Th12. Elevated protein level and lymphocytic pleocytosis were detected in the cerebrospinal fluid. A serologic blood test for SARS-CoV-2 showed recent infection. PCR for other viral infections was negative. The patient was treated with injectable steroids and showed full recovery. Specific neurological features of acute myelitis associated with COVID-19 were reported, described, and analyzed. Patient was treated and recovered.

KEY WORDS: COVID-19, SARS-CoV-2, myelitis, complication, infectious diseases, spinal cord

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INTRODUCTION

More and more data and evidence that confirm damage to the central nervous system (CNS) in the context of acute infection and post-infectious complications of COVID-19 have been reported [1-3]. It is essential to recognize the neurological manifestations and complications of COVID-19, some of which can progress rapidly and require urgent intervention.

COVID-19 DEFINITIONS

COVID-19 is a highly contagious respiratory disease in humans, caused by a new coronavirus called SARS-CoV-2. It was first identified in Wuhan city of China, in December 2019. [4] On February 11, 2020, the World Health Organization (WHO) announced an official name for the disease caused by the novel coronavirus – COVID-19. In this abbreviation, 'CO' stands for 'corona,' 'VI' for 'virus,' and 'D' for disease. Formerly, this disease was referred to as "2019 novel coronavirus" or "2019-nCoV." [5]

COVID-19 PANDEMIC HEALTH IMPACT

On March, 11, 2020, the WHO declared the ongoing outbreak of the respiratory disease, named Coronavirus disease 2019 (COVID-19), to be a global pandemic, pointing to over 3 million cases and 207,973 deaths in 213 countries and territories. [6] The morbidity and mortality of the global community due to this disease is increasing dramatically. As of January 11, 2021, authorities in 218 countries and territories around the world have reported over 90.8 million COVID-19 cases and 1.94 million deaths since China reported its first cases to the WHO in December, 2020. [7]

COVID-19 ECONOMIC IMPACT

The COVID-19 infection has not only become a public health crisis but has also affected the global economy. The pandemic caused the largest global recession in history, with more than a third of the global population at the time being placed on lockdown. [8]

The quarantines and lockdowns that are needed to fight the COVID-19's spread are freezing the economies with unprecedented force and speed. Significant economic impact has already occurred across the globe due to reduced productivity, premature deaths, business closures, workplace absenteeism, trade disruption, decimation of the tourism industry, and reduction in productivity. It has created a negative supply shock, with manufacturing productive activity slowing down due to global supply chain disruptions and closures of factories. [9] Worldwide, 660 million workers lost their jobs, 38 million have filed for unemployment insurance during the pandemic. A record 3.28 million Americans applied for unemployment benefits, the highest number ever recorded. [10]

The COVID-19 pandemic has triggered the deepest economic recession in nearly a century, threatening health, pushing the hospital system to its capacity, disrupting economic activity, and

hurting well-being and jobs. The pandemic has created a demand shock, a supply shock, and a financial shock all at once with the global labour income losses over US$3.5 trillion. [11, 12]

COVID-19 NEUROLOGICAL MANIFESTATIONS

As a second wave of COVID-19 occurred, it has become clear that SARS-CoV-2 may affect not only the respiratory system, but multiple parts of the body, including the nervous system. Reported neurological manifestations of COVID-19 include, but are not limited to: headache, impaired consciousness, stroke, seizure, meningitis, encephalitis, necrotizing encephalopathy, Guillain-Barré syndrome, and acute demyelinating encephalomyelitis [13-16].

Neurological complications in COVID-19 infected patients have not been widely reported. Here we report the case of a patient who presented with acute-onset non-compressive myelitis and was found to be infected with COVID-19.

The purpose of this study is to report, describe, and analyze a COVID-19-related case of acute myelitis in a young adult that has not been associated with any other viral infection. Comprehensive clinical and neuroimaging analysis follows.

CASE REPORT

A 23-year-old male student was admitted to the hospital with progressive bilateral lower extremity weakness, difficulty urinating, and constipation. A month earlier, he had a loss of taste (dysgeusia) and smell (anosmia). The symptoms disappeared on their own after 14 days. The patient was not examined for SARS-CoV-2, and therefore did not receive any treatment for COVID-19. On the 20th day from the time of loss of smell and taste, he developed weakness in his legs, and began to experience difficulty urinating. Over the next 7 days, sub-febrile conditions and pain in the lumbar and thoracic spine were noted. This was accompanied by progressive weakness of the lower extremities, difficulty walking, and constipation. He turned to the Regional Clinical Center of Neurosurgery and Neurology, Uzhorod city, Ukraine.

MEDICAL HISTORY

Patient does not smoke, does not take any recreational drugs or medications. No alcohol intake. No incidents of head or spinal injury. Denies chronic diseases and any surgical interventions. There is no family history of any neurological disorder.

VITALS UPON ADMISSION

Upon admission to the department, the patient showed a normal general condition. The respiratory rate was 16 breaths per minute, oxygen saturation was 100%, and blood pressure was 120/75 mm Hg. A heart rate of 70 beats per minute, temperature 36.7 ° C.

CLINICAL EXAMINATION

Clinical neurological examination revealed decreased muscle tone, hyporeflexia, and decreased proprioception of the lower extremities. Muscle strength in the legs was: 2.5 points on the left and 3.0 points on the right. The patient also had paresthesia on both sides from the Th9 level. No pathological reflexes were found. The patient was hospitalized for further examination and treatment.

LABORATORY AND INSTRUMENTAL EXAMINATION RESULTS

Preliminary general blood tests and X-Ray of the chest did not reveal any pathological changes. MRI of the spine showed a segment of increased T2 signal in the center of the spinal cord at the Th11-Th12 level (Figure 1). MRI of the brain and orbits did not reveal any signs of inflammatory changes to suggest a relationship with the changes in MRI seen in the spine.

Cerebrospinal fluid (CSF) showed increased protein level (1.02 g / L) and lymphocytic pleocytosis (130 cells). PCR for viral infections was negative for toxoplasma, herpes simplex viruses (types 1 and 2), and cytomegalovirus. SARS-CoV-2 (PCR) cerebrospinal fluid and oligoclonal bands were negative. A serologic blood test for SARS-CoV-2 showed recent infection (presence of SARS-CoV-2 / IgG as determined by internal immunofluorescence antibody testing). Antibodies to anti-myelin-associated glycoprotein IgM and IgG to optic neuromyelitis were negative in serum.

DIAGNOSIS

Based on initial diagnostic assessment (patient history, physical exam, evaluation of the patient's chief complaint and symptoms, differential diagnosis, and diagnostic tests and treatment results) patient was diagnosed with acute transverse myelitis at the level of Th11-Th12 (KOVID-19 associated), with lower moderate paraparesis, sensitive disorders, pelvic floor dysfunction (urinary retention and constipation), and gait disorders.

DIFFERENTIAL DIAGNOSIS

The presence of transverse sensorimotor myelopathy with segmental neurological deficit necessitated a detailed neurological examination. The patient did not report significant head or spinal trauma. The progressive appearance of neurological symptoms and young age made the diagnosis of cerebrovascular accident less likely. The patient's preliminary blood count and clinical examination did not indicate a serious systemic infection. The patient's medical history does not provide further indication of any cause for the disease. Neuroimaging, including brain imaging, showed no signs of a demyelinating disease such as multiple sclerosis or Devik's optic myelitis (taking into account, among other things, the absence of antibodies to aquaporin-4). Due to the indication of recent viral infection (COVID-19) in the form of its typical manifestations, and the presence of SARS-CoV-2 IgG in the blood, we assumed the possibility of developing post-viral acute partial transverse myelitis (ARTM).

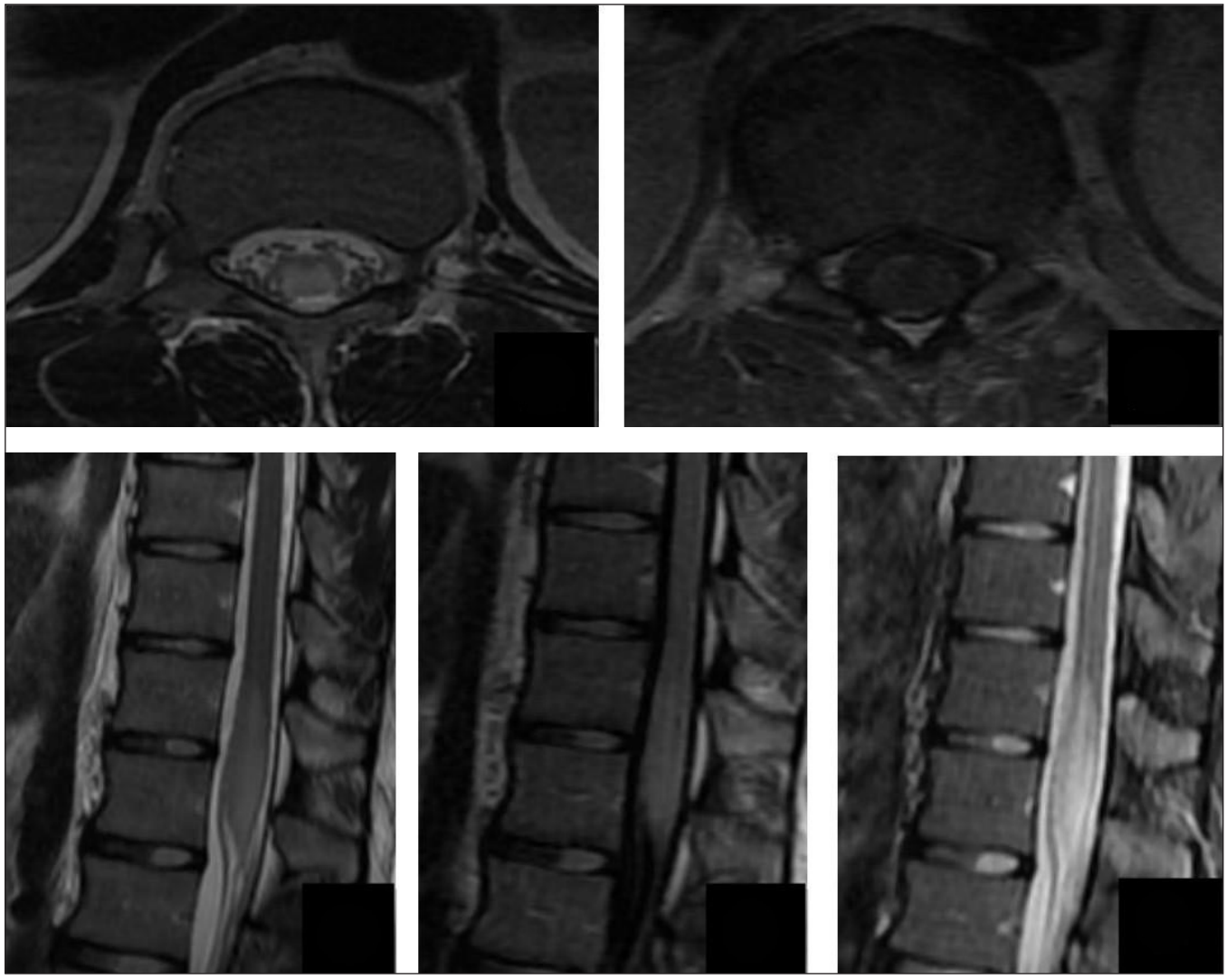

Fig 1. MRI of the patient's thoracic spine, sagittal projection (T2 FLAIR, STIR, and T2W sequences) and coronary projection (T2W).

TREATMENT

The patient was treated with methylprednisolone intravenous injections of 1 gram per day for 5 days. After that methylprednisolone was given in tablet form that was titrated, starting from 32 mg/day. No antiviral medicine, antibiotics, or immunomodulatory medicine, other than methylprednisolone, was prescribed. Neurological symptoms improved with corticosteroids treatment. The patient fully recovered motor strength in the lower extremities. Paresthesias disappeared completely and the pelvic disorders regressed. The patient was discharged home on the 8th day of hospitalization. Repeated MRI of the spinal cord was performed on day 30 and showed complete disappearance of intramedullary changes observed earlier at the Th 11-Th 12 level.

The incidence of acute myelitis associated with COVID-19 infection is not widely reported. Several reports of similar cases have been published in the literature, in which COVID-19 is associated with the onset of acute myelitis as a neurological complication. [17-21]

According to the literature, the most common symptoms associated with COVID-19 infection are fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, loss of taste or smell, headache, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea. [2, 24]. The symptoms may appear 2-14 days after exposure to the virus. [2, 24] It is believed that anosmia and dysgeusia are due to the fact that the SARS-CoV-2 virus can directly enter the nervous system via the olfactory tract or bloodstream at a pro-inflammatory stage of the disease. [1-3]

Acute necrotizing encephalopathy has been reported following the onset of the severe acute respiratory syndrome (SARS-CoV-2) and detection of viral DNA in a nasopharyngeal swab sample. [25] In this case, the analysis of cerebrospinal fluid was not performed for technical reasons, and the diagnosis was confirmed by neuroimaging.

Viral encephalitis has been reported as another manifestation of CNS damage in the context of acute COVID-19 infection. [26] In this case, SARS-CoV-2 RNA was found

in the cerebrospinal fluid, and the brain MRI results conformed to meningitis.

Cases of Guillain-Barré syndrome associated with COVID-19 [30], as well as polyneuropathy and Miller Fisher syndrome [32] have been reported. In the indicated cases, a nasal swab for SARS-CoV-2 PCR was positive. SARS-CoV-2 was not detected in the cerebrospinal fluid.

It was suggested a hypothesis that SARS-CoV-2 can cause neural damage through hypoxic and immune-mediated pathways. [2] SARS-CoV-2 binds to ACE2 receptors, which have been described in the heart, lungs, central nervous system, and skeletal muscle. [1,2] Viral replication and increased activation of the ACE2 receptor in the CNS can induce a systemic inflammatory response leading to increased permeability of the blood-brain barrier and immune-mediated CNS inflammation. IL-6, a pro-inflammatory cytokine, is thought to mediate this response. [27-29] Increased levels of IL-6 can lead to increased production of acute-phase proteins such as CRP and fibrinogen. [27]

It has recently been highlighted that IL-6 is a potential predictor of severity, progression, and mortality from COVID-19 infection. [27-29] Elevated ferritin levels have also been associated with more severe infection and worse outcomes. Unfortunately, the determination of the level of IL-6 and ferritin in our patient was not performed.

CONCLUSIONS

Health care providers should be aware that patients with COVID-19 can present with myelitis in the acute setting and during hospitalization. We presented a case of acute partial transverse myelitis in the context of a recent COVID-19 infection. We did not find SARS-CoV-2 RNA in the cerebrospinal fluid and suggested that this case was probably associated with an immune-mediated inflammatory process, and not the direct effect of SARS-CoV-2 on the central.

REFERENCES

1. Mao L., Jin H., Wang M. et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol. 20201;77(6):683-690. doi: 10.1001/jamaneurol.2020.1127.
2. Wu Y., Xu X., Chen Z. et al. Nervous system involvement after infection with COVID-19 and other coronaviruses. Brain Behav Immun. 2020;87:18-22. doi:10.1016/j.bbi.2020.03.031.
3. Asadi-Pooya A.A., Simani L. Central nervous system manifestations of COVID-19: A systematic review. J Neurol Sci. 2020;413:116832. doi:10.1016/j.jns.2020.116832.
4. Mackenzie J.S., Smith D.W. COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don't. Microbiol Aust. 2020;MA20013. doi:10.1071/MA20013.
5. Eurosurveillance editorial team. Updated rapid risk assessment from ECDC on the novel coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK. Euro Surveill. 2020;25(10):2003121. doi:10.2807/1560-7917.ES.2020.25.10.2003121.
6. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19—11 March 2020. www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020
7. Johns Hopkins Coronavirus Resource Center. https://coronavirus.jhu.edu/map.html.
8. Yousfi N., Bragazzi N.L., Briki W. et al. The COVID-19 pandemic: how to maintain a healthy immune system during the lockdown – a multidisciplinary approach with special focus on athletes. Biol Sport. 2020;37(3):211-216. doi:10.5114/biolsport.2020.95125.
9. Pak A., Adegboye O.A., Adekunle A.I. et al. Economic Consequences of the COVID-19 Outbreak: the Need for Epidemic Preparedness. Front Public Health. 2020;8:241. doi:10.3389/fpubh.2020.00241.
10. Rothwell J. The effects of COVID-19 on international labor markets: An update. Middle Class Memos. 2020. https://www.brookings.edu/research/the-effects-of-covid-19-on-international-labor-markets-anupdate.
11. ILO Monitor: COVID-19 and the world of work. Sixth edition. https://www.ilo.org/wcmsp5/groups/public/---dgreports/---dcomm/documents/briefingnote/wcms_755910.pdf
12. ILO Annual Meetings of the World Bank and the IMFGovernment economic and social policy interventions vital to tackle workplace closures and labour income losses. https://www.ilo.org/global/about-the-ilo/newsroom/statements-and-speeches/WCMS_758304/lang--en/index.htm
13. Koralnik I.J., Tyler, K.L. COVID–19: A Global Threat to the Nervous System. Ann Neurol. 2020; 88: 1-11. doi:10.1002/ana.25807.
14. Mao L., Jin H., Wang M. et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol. 2020;77(6):683–690. doi:10.1001/jamaneurol.2020.1127.
15. Moriguchi T., Harii N., Goto J. et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis. 2020;94:55-58. doi:10.1016/j.ijid.2020.03.062.
16. Poyiadji N., Shahin G., Noujaim D. et al. COVID-19-associated Acute Hemorrhagic Necrotizing Encephalopathy: Imaging Features. Radiology. 2020;296(2):E119-E120. doi: 10.1148/radiol.2020201187.
17. Zhao K., Huang J., Dai D. et al. Acute myelitis after SARS-CoV-2 infection: a case report. medRxiv 2020. doi:10.1101/2020.03.16.20035105.
18. Sarma D., Bilello L.A. A Case Report of Acute Transverse Myelitis Following Novel Coronavirus Infection. Clin Pract Cases Emerg Med. 2020;4(3):321-323. doi:10.5811/cpcem.2020.5.47937.
19. Munz M., Wessendorf S., Koretsis G. et al. Acute transverse myelitis after COVID-19 pneumonia. J Neurol. 2020;267(8):2196-2197. doi:10.1007/s00415-020-09934-w.
20. Chow C.C.N., Magnussen J., Ip J., Su Y. Acute transverse myelitis in COVID-19 infection. BMJ Case Rep. 2020;13(8):e236720. doi:10.1136/bcr-2020-236720.
21. Valiuddin H., Skwirsk B., Paz-Arabo P. Acute transverse myelitis associated with SARS-CoV-2: A Case-Report. Brain Behav Immun Health. 2020;5:100091. doi:10.1016/j.bbih.2020.100091.
22. Palahuta H.V., Yevtushenko S.K. A case of acute myelitis as a neurological complication of COVID-19. International Neurological Journal. 2020;7(16):32-35. (in Ukrainian). DOI: https://doi.org/10.22141/2224-0713.16.7.2020.218244.
23. Fartushna O.Y., Palahuta H.V., Yevtushenko S.K. Neurological and neuropsychiatric manifestations and complications of SARS–CoV–2 infection: a narrative review and a case presentation in a previously healthy young white adult. International Neurological Journal. 2021;1(17):12-16.
24. Phua J., Weng L., Ling L. et al. Asian Critical Care Clinical Trials Group. Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. Lancet Respir Med. 2020;8(5):506-517. doi: 10.1016/S2213-2600(20)30161-2.

25. Poyiadji N., Shahin G., Noujaim D. et al. COVID-19-associated Acute Hemorrhagic Necrotizing Encephalopathy: Imaging Features. Radiology. 2020;296(2):E119-E120. doi: 10.1148/radiol.2020201187.
26. Moriguchi T., Harii N., Goto J. et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis. 2020;94:55-58. doi: 10.1016/j.ijid.2020.03.062.
27. Zhu Z., Cai T., Fan L. et al. Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. Int J Infect Dis. 2020;95:332-339. doi: 10.1016/j.ijid.2020.04.041.
28. Zhou F., Yu T., Du R. et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3.
29. Ruan Q., Yang K., Wang W. et al. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020;46(5):846-848. doi: 10.1007/s00134-020-05991-x.
30. Zhao H., Shen D., Zhou H. et al. Guillain-Barré syndrome associated with SARS-CoV-2 infection: causality or coincidence? Lancet Neurol. 2020;19(5):383-384. doi: 10.1016/S1474-4422(20)30109-5.
31. Gutiérrez-Ortiz C., Méndez-Guerrero A., Rodrigo-Rey S. et al. Miller Fisher syndrome and polyneuritis cranialis in COVID-19. Neurology. 2020;95(5):e601-e605. doi: 10.1212/WNL.0000000000009619.

ORCID and contributionship:
Hanna V. Palahuta: 0000-0001-7348-4390 [A,B,D,E,F]
Olena Y. Fartushna: 0000-0002-4641-0836 [A,B,D,E,F]
Stanislav K. Yevtushenko: 0000-0003-2528-716X [A,E,F]
Yana Y. Hnepa :0000-0002-0276-1466 [E,F]

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Olena Y. Fartushna
Ukrainian Military Medical Academy
24 Melnikova St., 04050 Kyiv, Ukraine
tel: +380935488978
e-mail: olena.y.fartushna@gmail.com

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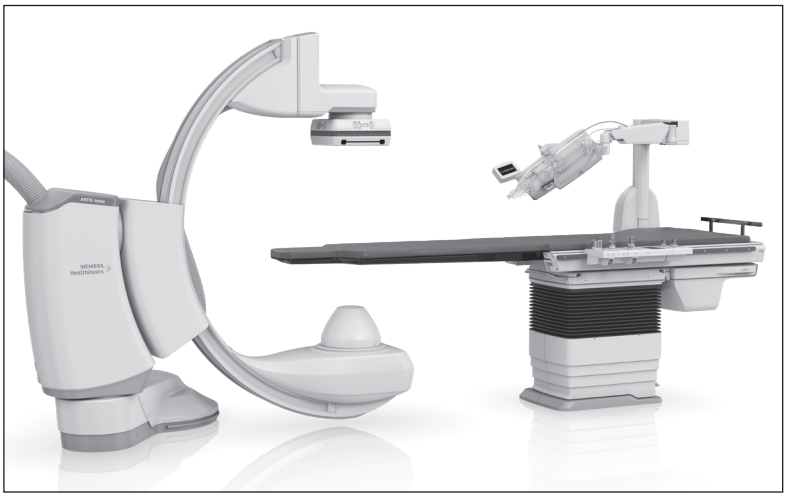
CORINDUS, A SIEMENS HEALTHINEERS COMPANY

The company has invested heavily in research and development to build next generation capabilities to transform care while expanding commercial capabilities to provide the benefits of vascular robotics to patients and hospitals. Corindus is focused on developing innovative robotic solutions, such as remote robotics and advanced automation, to revolutionize treatment of emergent conditions such as heart attack and stroke. Remote robotics, or telerobotics, could potentially reduce time to treatment, provide greater access to highly skilled specialists and increase the number of treated patients, especially in stroke treatment. By incorporating additional advanced capabilities such as automation and integration with imaging systems, the company also aims to standardize treatment algorithms, increase efficiency, and improve patient care.



What is CorPath Robotic Angioplasty?

CorPath Robotic Angioplasty combines the benefits of coronary angioplasty with the precision of robotics, offering many benefits to patients, including roboting precision in positioning stents.

With the CorPath GRX System, your doctor can move your stent a millimeter at a time. The length of your anatomy can also be measured by your doctor to help choose the right stent for you. The CorPath GRX System enables your doctor to have an enhanced, close up view of the angiographic images and information for the entire procedure.

Using the CorPath robotic techniques, your doctor can precisely advance your stent to the blocked vessel, potentially leading to a shorter procedure and less exposure to harmful radiation. Think of the robot as an extension of your doctor's hands, affording robotic precision and detailed visualization of the positioning of guide catheters, guidewires, and balloon/stent catheters.

CorPath GRX is the second generation robotic system from Corindus. During a robotic-assisted intervention, the physician sits down in a radiation protected workstation and uses joysticks to manipulate devices that are held in a robotic arm located on the patient table. In the US, CorPath GRX is indicated for use by the FDA in coronary and peripheral vascular interventions and the company is working to expand its indication to include neurovascular interventions.

The team of Polish cardiologists in Nowy Sącz has performed the first invasive cardiology procedures with the support of a robot in Central and Eastern Europe. The Corindus CorPath GRX system is the only device of this type with the CE mark and approved by the American FDA. The implementation of robotization has revolutionized the organization of the interventional cardiology laboratory, significantly increasing the precision and effectiveness of procedures as well as the safety of the medical team's work.

(Press Release)