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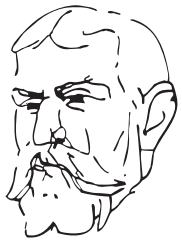

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ORIGINAL ARTICLE

PECULIARITIES OF THE ORBIT MORPHOGENESIS AT AN EARLY PERIOD OF HUMAN ONTOGENESIS

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ABSTRACT

The aim: To clarify the sources and determine the chronological sequence of the germs of anatomical structures of the orbit at the early period of human ontogenesis.

Materials and methods: Using a complex of methods of morphological examination (morphometry, microscopy, three-dimensional computer reconstruction and statistical analysis) 30 series of consecutive histological sections of human embryos and prefetuses aged from 3 till 8 weeks od IUD (3.0-30.0 mm parietal-coccygeal length (PCL)) were studied.

Results: At the 4rd week of IUD the orbital region is represented by the place of close contact of the neuroectoderm of the optic vesicle with the adjacent integumentary ectoderm, as well as the mesenchyme surrounding this place of contact. In embryos of 4.0 mm PCL, the rudiment of the optic stalk is observed as a result of the transformation of the junction of the area of the eye rudiment with the brain.

Conclusions: 1. The rudiments of the organ of vision (lens placodes) appear in the 4rd week of IUD. At the 5th week of IUD as a result of gradual intussusception of crystalline placodes into the adjacent mesenchyme, lens pit are formed, and then – lens vesicles. 2. Rudiments of the extraocular muscles (except for the inferior oblique muscle) was detected at the end of the 5th week of IUD. The rudiment of the inferior oblique muscle develops from a single mesodermal islet located in the mesenchyme medially and below the eyeball. Simultaneously with the rudiments of the extraokular muscles, the trochlear and abductor nerves are develop and ingrown into the orbit. 3. The development of blood vessels of the orbit occurs from two sources – from the islands of local angiogenesis, which begins in the 5th week of IUD, and from extraorganic vessels, which can be traced in the form of a vascular network at the end of the 6th week of IUD. The combination of both sources is observed at the end of the 7th week of IUD. 4. The embryonic period of ontogenesis is the first critical period in the development of the human orbit, due to the formation of the muscles, nerve and vascular structures.

KEY WORDS: orbit, embryo, prefwtus, prenatal ontogenesis, human

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INTRODUCTION

Finding out the sources, the chronological sequence of the germs and the formation of the structure of the human orbit remains a important task for morphologists. Eye diseases in 85.3% of cases are congenital or acquired in childhood [1, 2]. One of the main causes of strabismus, amblyopia, binocular vision disorders, myopia and astigmatism is the pathology of the eyeball muscles [3]. Clinicians have repeatedly emphasized that scientific studies of age-dependet morphological features of the organ of vision are clearly insufficient [4]. In addition, the development and application of new microsurgical operations require a deeper knowledge of the surgical anatomy of the orbit [5]. Anatomical data on the development and formation of topography of the vascular-nervous and muscular structures of the human eyeball are fragmentary and do not give a clear data of the sequence of structural changes during intrauterine development (IUD) [1, 3]. A comprehensive study of the features of development, formation, topographic and anatomical changes in the structures of the orbit (in particular, the muscles of the eyeball, blood vessels and nerves), the dynamics of their syntopic changes during the erly prenatal period of ontogenesis is important to determine the structure, preconditions and time of occurrence of their congenital malformations [6, 7].

THE AIM

To clarify the sources and determine the chronological sequence of the germs of anatomical structures of the orbit at the early period of human ontogenesis.

MATERIALS AND METHODS

We have examined 30 series of consecutive histological sections of human embryos and prefetuses aged from 3 till 8 weeks of IUD (3.0-30.0 mm parietal-coccygeal length (PCL)). The material was obtained and studied at Chernivtsy Regional Pathologists Office (Ukraine) in accordance with bilateral collaboration with the Department of Histology, Cytology and Embryology. In order to visualize necessary structures of the orbit we have used complex of

methods of morphological investigation: morphometry, microscopy, 3D-reconstruction and statistical analysis. The study was performed in accordance with the provisions of the Declaration of Helsinki on ethical issues of studies conducted with humans (1964-2008). All specimens were obtained from ectopic pregnancies or spontaneous abortions, and no part of the material gave indications of possible malformation. Approval for the study was granted by the Ethics Committee of the Bukovinian State Medical University.

RESULTS

To development of the orbit region with the organ of vision precedes the process of differentiation of the rudiment of the anterior part of the neural tube from three cerebral vesicles (anterior – forebrain, middle – midbrain and posterior – rhombencephalon) into five due to the separation of the middle and posterior cerebral vesicles. The anterior cerebral vesicles forms the diencephalon and the telencephalon. The stage of the five-vesicles rudiment of the brain is clearly observed at the beginning 3rd weeks of IUD. The sources of the eyeball rudiment are traced at the beginning of the 4rd week of IUD (embryos of 4.0 and 4.3 mm PCL), when the lateral protrusions of the neuroectoderm of the diencephalon (optic vesicles) reach the ectodermal cover of the embryo (Fig. 1). During this period, the orbital region is represented by the place of close contact of the neuroectoderm of the optic vesicle with the adjacent integumentary ectoderm, as well as the mesenchyme surrounding this place of contact.

In embryos of 4.0 mm PCL, the rudiment of the optic stalk is observed as a result of the transformation of the junction of the area of the eye rudiment with the brain (Fig. 2).

In embryos of 4.0-5.0 mm PCL (4th week of IUD) structural transformations of tissues at the point of contact of the nervous tissue of the eyeball and the ectoderm of the lens placode are observed. Nerve tissue continues to protrude into the adjacent mesenchyme in the direction of the ectoderm, and the ectoderm of the lens placode thickens, invaginates the adjacent neuroectoderm, which leads to the formation of lens pits and the beginning of formation the optic cups (Fig. 3).

The mesenchyme surrounds the vesicles and the optic stalk, and extends from the brain to the ectodermal covering of the cranial part of the embryo. In human embryos of 4.0 and 5.0 mm PCL (4th week of IUD) observed a condensation of the mesenchyme in the rudiment of the orbital region, it localized around the junction of the optic stalk with the cerebral vesicle. We believe that this condensation of the mesenchyme in the form of a membranous plate, close to the rudiment of the brain, has no direct anatomical relation to the organ of vision, and is the rudiment of the ectomeningeal capsule.

In embryos of 7.0 and 7.5 mm PCL (5th week of IUD) there is a gradual intussusception of lens placodes into the adjacent mesenchyme, which leads to their transformation into lens pit, and then – in the lens vesicle. The lens vesicles begin to untie from the integumentary epithelium of the head, close and turn into epithelial bodies of round shape, adjacent to the optic vesicles. Their wall is invaginated, as a result of which they gradually turn into double-walled cups. The optic cups are connected to the cavity of the anterior cerebral vesicle by means of the optic stalk. Both optic cups and optic stalks are in the cell mass of the mesenchyme. Subsequently, the lens placoda forms the lens of the eye, while the optic cup forms the retina and other parts of the eye (Fig. 4).

The sources of the extraocular muscles (except for the inferior oblique muscle) was first detected at the end of the 5th week of IUD, when in embryos 7.0-7.5 mm PCL in the adjacent mesenchyme behind the optic cups and around the optic stulks appear the foci of condensation of mesodermal cells of irregular elongated shape (Fig. 5). The rudiment of the inferior oblique muscle develops from a single mesodermal islet located in the mesenchyme medially and below the eyeball.

3D-reconstruction revealed the general mesodermal rudiment of the eyeball muscles, its shape and the beginning of differentiation of each individual muscle from the mesoderm rudiment, which has a funnel-shap and covers the optic stalk. Its thickened end is directed towards the eyeball and ends in front with five small protrusions. These protrusions, as shown by studies of microspecimens of older age groups (prefetal period of IUD), are a morphological substrate for the development of individual muscles of the eyeball (Fig. 6). In addition, the mesodermal rudiment is the basis for the formation of a common tendon ring of the proximal ends of the eye muscles. The inferior oblique muscle develops from a single mesodermal rudiment and therefore has a different location and fixation points.

Simultaneously with the rudiments of the extraocular muscles, the trochlear and abductor nerves are develop and ingrown into the orbit. In the embryonic period, we noted a fairly large diameter of these nerves relative to the small thickness of the rudiments of the muscles (Fig. 7). Later, in the process of further development, there is a gradual predominance of the growth of the extraocular muscles relative to the nerves that provide their innervation.

In the cell mass of the mesenchyme of embryos of 7.0-7.5 mm PCL, which surrounds the eyes and mesodermal sources of the muscles, form chains of islets of intraorganic hematopoiesis and cells such as erythroblasts. Part of the islets of intraorganic hematopoiesis is separated from the adjacent mesenchyme by a number of elongated endothelial cells. In embryos of 13.0-16.0 mm PCL there is a vascular network in the orbital region (Fig. 8). It is during this period that the differentiation of the individual extraocular muscles begins.

In addition, the muscular connecting branches of extraorganic vessels grow into the eye muscles. The combination of both parts of the vascular formations occurs in the prefetuses 18.0-22.0 mm TCL. Thus, the vascular supply to the extraocular muscles is formed in two ways – in the form of islets of intra-organ hematopoiesis and ingrown muscle branches of extra-organ vessels.

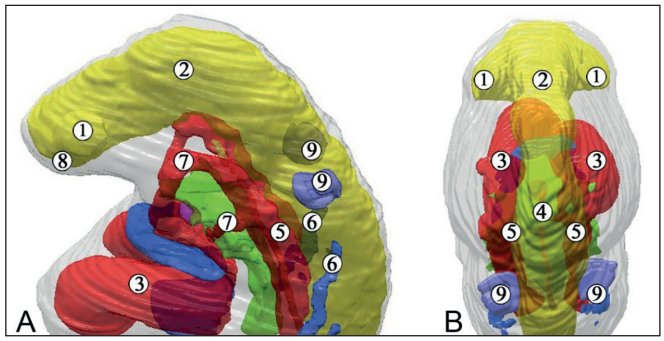

Fig. 1. 3D-reconstruction of the upper half of the human embryo 4.0 mm PCL. Left lateral (A) and superior posterior (B) projections. Magn.: x20: 1 – optic vesicle; 2 – brain; 3 – heart; 4 – notochord; 5 – dorsal aorta; 6 – cardinal vein; 7 – aortic arches; 8 – frontal process; 9 – otic vesicle.

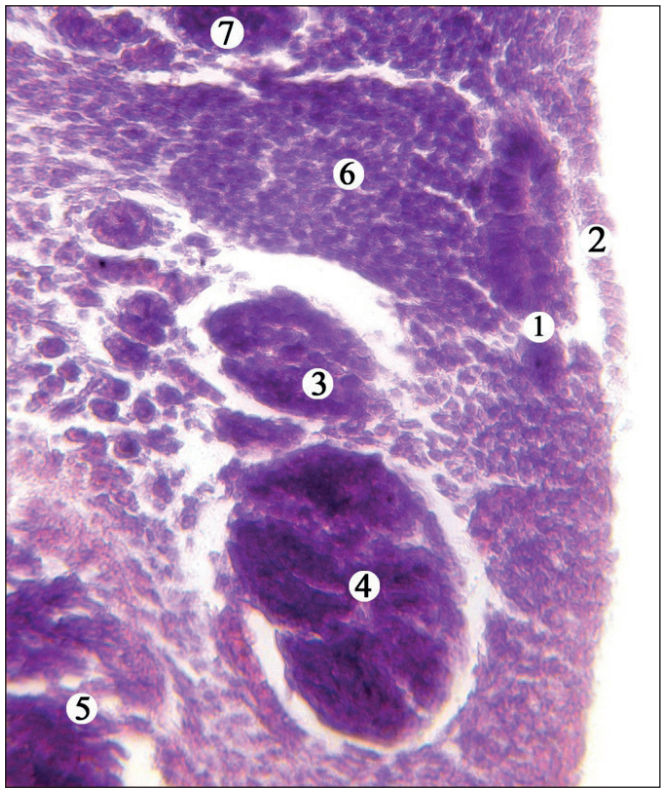

Fig. 2. Frontal histological section of the cranial end of the human embryo 3.5 mm PCL (3rd week of IUD) (right half). Hematoxylin and eosin staining. Microphotograph. Magn.: x200: 1 – neuroectoderm of the optic vesicle; 2 – ectodermal lens placode; 3 – VII cranial nerve; 4 – VIII cranial nerve; 5 – neuroectoderm of the midbrain; 6 – optic stalk; 7 – neuroectoderm of the telencephalon.

DISCUSSION

In our study by means of a complex of morphological methods, the development of the structures of the orbit and the organ of vision successively in the age-related dynamics of prenatal development has been studied. The method of 3D reconstruction of a series of consecutive histological sections of the head and microscopic examination of our material was widely used.

The majority of scientific studies of prenatal development of the human organ of visus is limited to a certain age

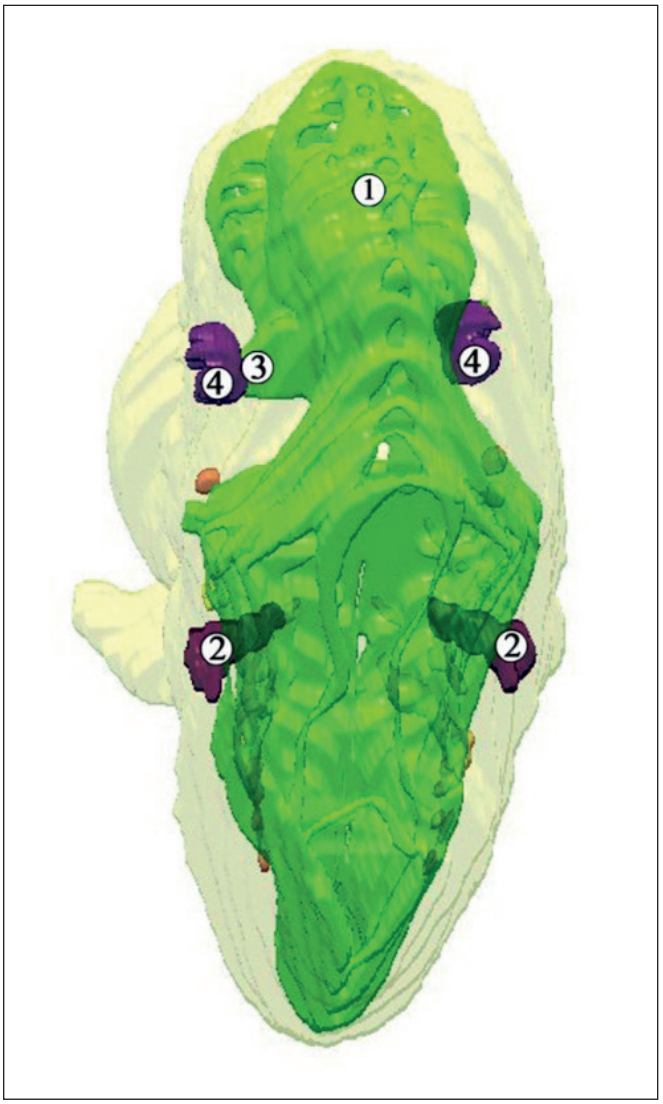

Fig. 3. 3D-reconstruction of the upper half of the human embryo 5.0 mm PCL. Superior posterior projection. Magn.: x20: 1 – brain; 2 – otic vesicle; 3 – optic stalk; 4 – optic cups.

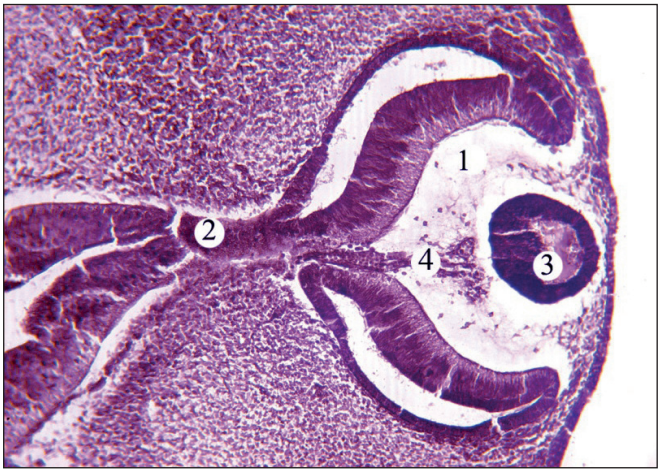

Fig. 4. Frontal section of the left cranial part of the embryo 9.0 mm PCL (6th week of IUD). Hematoxylin and eosin staining. Microphotograph. Magn.: x40: 1 – the rudiment of the eyeball; 2 – optic stulk; 3 – lens vesicle; 4 – hyaloid artery.

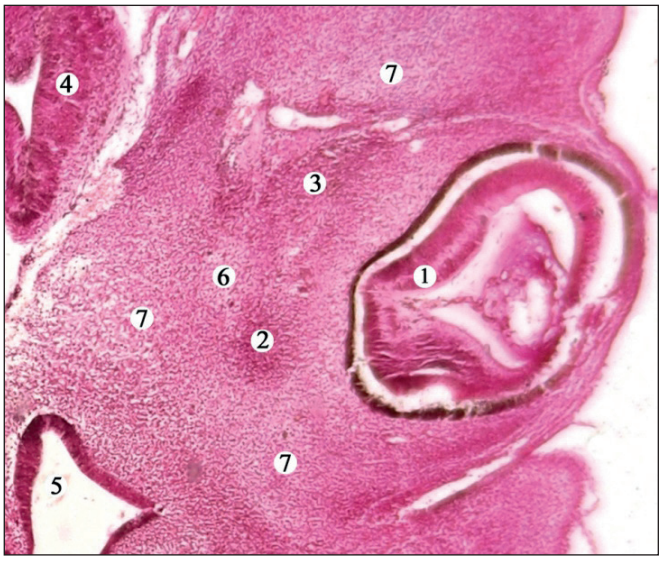

Fig. 5. Frontal section of the left side of the human embryo head 7.0 mm PCL. Hematoxylin and eosin staining. Photomicrograph. Magn.: x60: 1 – eye; 2 – the rudiment of the lateral rectus muscle; 3 – the rudiment of the superir rectus muscle; 4 – brain; 5 – the left upper nasal conch; 6 – the rudiment of the optic nerve; 7 – mesenchyme of the head.

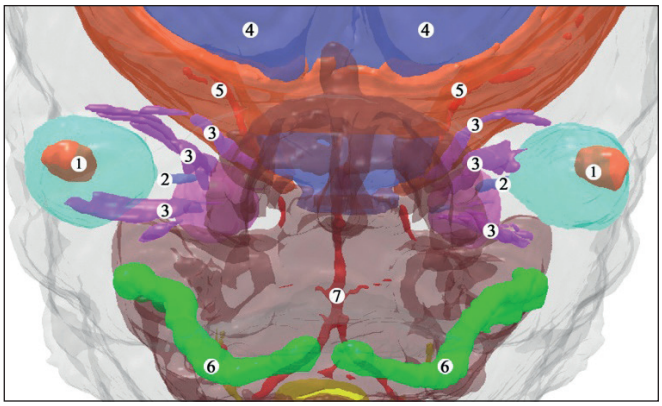

Fig. 6. 3D-reconstruction of the human prefetus head 13.5 mm PCL. Front projection. Magn.: x25: 1 – the rudiments of the eyeballs; 2 – optic nerve; 3 – the rudiments of the extraocular muscles; 4 – hemispheres of the brain; 5 – the rudiment of skull bones; 6 – Meckel's cartilage; 7 – basilar artery.

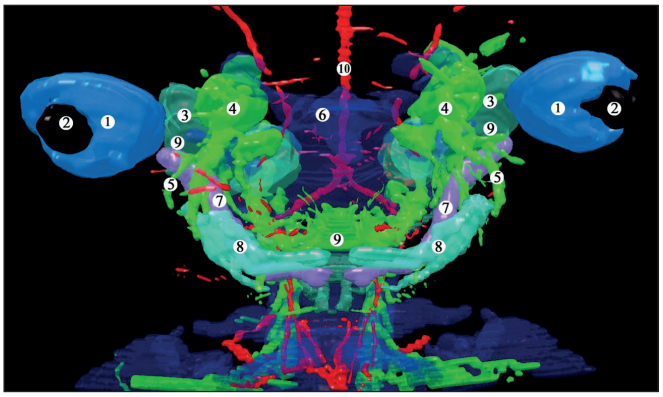

Fig. 7. 3D-reconstruction of the human prefetus head 17.0 mm PCL. Front projection. Magn.: x25: 1 – eyeballs; 2 – lens; 3 – optic nerve; 4 – trigeminal nerve; 5 – facial nerve; 6 – the rudiments of skull bones; 7 – Meckel's cartilage; 8 – the rudiment of the mandible; 9 – spine; 10 – artery.

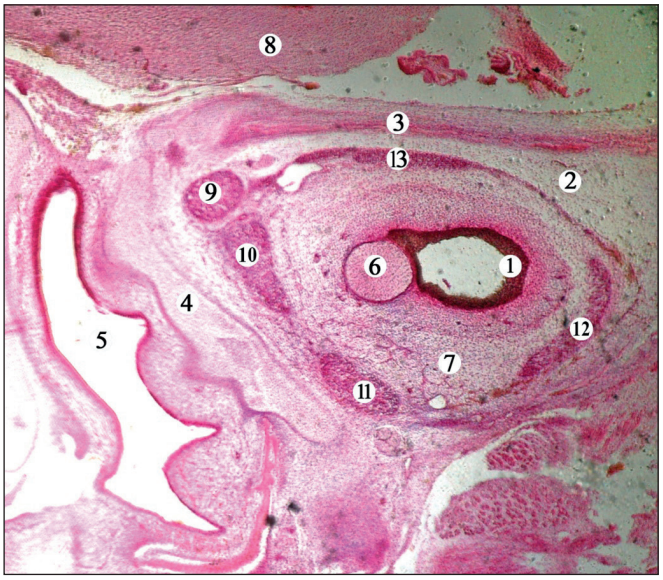

Fig. 8. Frontal section of the left half of the human embryo head 16.0 mm PCL. Hematoxylin and eosin staining. Photomicrograph. Magn.: x60: 1 – pigment epithelium; 2 – mesenchyme of the orbit; 3 – rudiment of the frontal bone; 4 – rudiment of the lacrimal bone; 5 – nasal cavity; 6 – optic nerve; 7 – blood vessels of the orbit; 8 – brain; 9 – superior oblique muscle; 10 – medial rectus muscle; 11 – inferior rectus muscle; 12 – lateral rectus muscle; 13 – upper rectus muscle.

period. We studied the development of the orbit and the eye from its source (embryos 3-4 weeks IUD), and to the beginning of the prenatal period (8th week IUD), when we can talk about the presence of all structures of the organ of visus (including blood vessels, nerves, muscles) in almost definitive topographic and anatomical relations.

The question of the time and sequence of appearance of germs of the main structures of the human organ of visus remains debatable. Thus, according to our data, the optic pits are formed in the form of depressions on both sides of the neural tube in the forebrain, even before the complete closure of the neural tube. At the beginning of the 4th week of IUD, after closing the neural tube, the optic pits form lateral optical vesicles. According to Cook C.S. [8], this process occurs on the 25th day, which corresponds to the middle of the 4th week of IUD. Some differences in the time of appearance of sources can be explained by an error in the methods of determining the age of the embryo. During the 4th week of IUD, the optic vesicles are initially in contact with the superficial ectoderm, but are gradually separated from it by cells originating from the neural crest and mesoderm. The cells of the neural crest and the mesoderm together form the mesenchyme, from which the connective tissue of the eyeball and the orbit develops. It is difficult to determine the origin of a certain structure – from the nerve crest or from the mesoderm, by classical morphological methods, because mesodermal cells and cells of the nerve crest have the same cytological appearance.

A number of successive processes of structural transformation of the rudiment of the organ of visus leads to the

beginning of the formation of the optic cup and optic stalk at the 5th week of IUD, which ends with the formation of a two-layer eye cup at 7th week of IUD [9].

The mesenchyme proliferates and migrates around the optic cup, and subsequently differentiates into the connective tissue of the orbit. The cells of the neural crest form the stroma of the cornea and endothelium, the choroid plexus and melanocytes, the ciliary muscle, most of the sclera, connective tissue and meningeal tunic of the optic nerve, connective tissue of the eyelids, conjunctiva and orbit. There is no doubt that the vascular endothelium and striated muscles are formed from the mesoderm [10]. We do not agree with Mann I. [11] that the external muscles of the eye develops gradually – first behind, at the apex of the orbit, from where they gradually grow frontward. We agree with the statement of Barishak Y.R. [10], Sevel D. [12] on the almost simultaneous development of extraocular muscles and their structural components. According to them, the muscles innervated by the III pair of cranial nerves originate from the I pair of somites on about the 26th day of IUD. The lateral rectus muscle, innervated by the VI pair of cranial nerves, develops from the mesenchyme of the maxillar and mandibular regions on about day 27 of IUD. The anterior oblique muscle, innervated by the IV pair of cranial nerves, originates from the II pair of somites on day 29 of IUD. This is broadly consistent with our results on the sources of muscles in embryos in the middle of the 5th week of IUD.

CONCLUSIONS

1. The rudiments of the organ of vision (lens placodes) appear in the 4rd week of IUD. At the 5th week of IUD as a result of gradual intussusception of crystalline placodes into the adjacent mesenchyme, lens pit are formed, and then – lens vesicles.
2. Rudiments of the extraocular muscles (except for the inferior oblique muscle) was detected at the end of the 5th week of IUD. The rudiment of the inferior oblique muscle develops from a single mesodermal islet located in the mesenchyme medially and below the eyeball. Simultaneously with the rudiments of the extraokular muscles, the trochlear and abductor nerves are develop and ingrown into the orbit.
3. The development of blood vessels of the orbit occurs from two sources – from the islands of local angiogenesis, which begins in the 5th week of IUD, and from extraorganic vessels, which can be traced in the form of a vascular network at the end of the 6th week of IUD. The combination of both sources is observed at the end of the 7th week of IUD.
4. The embryonic period of ontogenesis is the first critical period in the development of the human orbit, due to the formation of the muscles, nerve and vascular structures.

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ORIGINAL ARTICLE

A PROSPECTIVE STUDY TO ANALYZE THE SPECIFICITY OF CHLAMYDIAL HEAT SHOCK PROTEIN (CHSP60) ANTIBODIES TO DIAGNOSE TUBAL INFERTILITY

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ABSTRACT

The aim: To investigate the utility of testing for chlamydial heat shock protein 60 (CHSP60) antibodies in the diagnosis of tubal infertility_

Materials and methods: All the collected samples were assayed for IgM and IgG antibodies to chlamydia trachomatis and chlamydial heat shock protein 60 (CHSP60) by using immunofluorescence and enzyme-linked immunosorbent assay (ELISA) techniques, respectively.

Results: There were no substantial differences between antibodies to *C. trachomatis* in females with tubal infertility (67%) and non-tubal infertility (48%). However, women with tubal infertility (45%) have more anti-CHSP60 antibodies than non-tubal infertility (9%). Antibody screening for *C. trachomatis* has only (63%) sensitivity and (54%) specificity for detecting tubal infertility. On the other hand, the CHSP60 antibody testing has (44%) sensitivity and 92% specificity for diagnosing tubal infertility. A positive microimmunofluorescence (MIF) titer was observed in 12 of 18 (67%) females with the tubal problem, 31 of 64 (48%) with non-tubal infertility (P=0.3, OR=2.2, 95% CI=0.71 to 8.01). The CHSP60 antibodies were found in 8 of 18 (45%) females with tubal problem & 6 of 64 (9%) women with non-tubal infertility, power factor alpha α P=0.004, OR=9.3, 95% CI=2.1 to 43.2, power= 1.002 for n= 0.05). Incorporating CHSP60 and *C. trachomatis* antibodies testing gives an excellent positive probability proportion of 10 to diagnose *C. trachomatis* associated tubal infertility.

Conclusions: CHSP60 antibody testing is a more specific evaluation than antibody testing for *C. trachomatis* for predicting chlamydia-associated tubal infertility. Using these tests at the first infertility examination may help the immediate diagnosis for non-interceptive tubal infertility.

KEY WORDS: tubal infertility, tubal occlusion, chlamydial heat shock protein (CHSP60), *Chlamydia trachomatis*, antibodies

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INTRODUCTION

Infection with *Chlamydia trachomatis* is an important sexually transmitted condition related to tubal infertility with increased salpingitis episodes that leads to tubal blockage [1-3]. Many studies revealed that serologic evidence of previous *Chlamydia* infection is exceptionally related to tubal infertility [4-8] and also decreased the success rate of a positive outcome for in vitro fertilization (IVF) [9]. The individuals with positive serology to chlamydia are at higher risk of developing the pelvic inflammatory disease (PID) [10, 11].

It was suggested that chlamydial antigen might trigger the pro-inflammatory response in host immune cells [12]. The oxidative damage to the DNA and decreased antioxidants concentration may be associated with chlamydia-induced tubal damage [13]. The immunopathology seen in genital tract infections is similar to those elicited by the chlamydial conjunctival infection resulting in scarring trachoma. The reinfection is determined as an essential risk factor in the pathogenesis of trachoma development [14].

The mechanism by which chlamydial infection results in tubal damage have been studied however stays uncertain. Primate research studies recommend that reinfection with *C. trachomatis* may be the essential part of chronic salpingitis, causing distal tubal blockage [15].

The serological analysis and diagnosis for chlamydial antibodies early point can be a prognostic tool to estimate the risk of CT-related complications and prevent late complication development. Recent research studies have shown a strong association between antibody action to the chlamydia heat shock protein 60 (CHSP60) and ectopic pregnancy [16]. Furthermore, seropositivity to human HSP60 decreases the chance for ectopic gestation in individuals with previous chlamydial infection [17], along with the advancement of chlamydia-associated tubal infertility [18].

Chlamydial heat shock protein is a homolog of the groEL family of heat shock proteins [19, 20]. This family of proteins is highly conserved among both eukaryotes and prokaryotes [21, 22]. It has been recommended that antibodies versus conserved epitopes on CHSP60 might cross respond with those of hHSP60 and initiate an autoimmune inflammatory response [23-27].

Table 1. Summary of individuals included in this study

SR No	Pathology	Sample size (n=82)
1	Tubal infertility	Age= 34.2 ± 2.4 years, (n=18)
2	Non tubal infertility	Age= 32 ± 5 years, (n=64)
	Associated with:	
I	Male oriented	14
II	Ovulatory Dysfunction	16
III	Combined Male And Ovulatory Dysfunction	18
IV	Idiopathic	17

A research study identified serum antibodies testing to the entire *C.trachomatis* organism as more precise than hysterosalpingography (HSG) to predict tube-related infertility [5]. We compared chlamydia serology and antibodies accuracy versus CHSP60 in predicting infertility diagnosis related to tubal factors in a prospective study.

THE AIM

This study is aimed to investigate the effectiveness of chlamydial heat shock protein 60 (CHSP60) antibodies test and to analyze the specificity and sensitivity of CHSP60 measured by ELISA in comparison with standard chlamydial antibodies measurement with other diagnostic techniques like microimmunofluorescence (MIF) in the diagnosis of tubal infertility in a prospective study.

MATERIALS AND METHODS

After the suggested research study with permission approved by the Study Ethics Board at the Obstetrics and gynecology post-graduation department, Bogomolets national medical university, Kyiv, Ukraine, sample were obtained from 82 unselected females presenting for first infertility examination in the infertility center at the gynecology department, Bogomolets national medical university, Kyiv, Ukraine. All women had necessary examinations performed, consisting of basal body temperature charting, and/or mid-luteal, and/or late luteal endometrial biopsy, cervical *C. trachomatis* screening with ELISA or polymerase chain reaction assays. The medical diagnosis of tubal infertility made by HSG and/or laparoscopic test exposing distal tubal blockage or laparoscopic evidence of peritubular adhesions. A laparoscopic assessment was not performed if the complete reciprocatory distal tubal obstruction was diagnosed on HSG. All collected samples were assayed for immunoglobulin IgG and IgM antibody to *C. trachomatis* by the microimmunofluorescence (MIF) approach of Wang, also Grayston [28] utilizing detoxed Formalin-fixed primary bodies.

Sera were assessed at a dilution of 1:8 and were tittered at double dilutions to the endpoint. Chlamydia trachomatis seropositivity was defined as a MIF titer of n 1:8 [29]. An ELISA utilizing recombinant CHSP60 expressed as a mixed protein with glutathione-S-transferase, as an antigen, was used to analyze antibodies existence to CHSP60 as described previously [18, 30] patient sera were diluted 1:500 in addition to incubated with recombinant antigen bound to 96-well microtiter plates. Horseradish peroxidase-conjugated goat anti-human IgG was added, and the optical density of each was well determined. All sera favorable by ELISA were confirmed by immunoblotting using recombinant CHSP60 as antigen. Sera were checked blindly and without the information of clinical diagnose.

Groups were contrasted by y2 or Fisher's exact test. Odds ratios (OR) with 95% confidence intervals (CI) calculated probability ratios are also computed to help the diagnostic precision of both serologic tests for their ability to anticipate tubal disease as the reason for infertility the patients came for infertility assessment. The computation of possibility ratios makes it possible to compare the diagnostic importance of tests independent of infection frequency in varying populations. The ratio for positive test calculated as sensitivity/(100 — specificity). A positive possibility of 2 to 5 recommends a non-satisfactory clinical test, 5 to 10 shows a good scientific test, and > 10 is an excellent clinical test. The ratio for the negative test was also calculated as sensitivity/(100 — specificity). The unfavorable ratio of 0.5 to 0.2 shows an unsatisfactory examination, 0.2 to 0.1 is a good clinical assessment, and value <0.1 shows a superb clinical assessment [31].

RESULTS

Eighty-two (82) women were included in our research, 18 with the final diagnosis of tubal disease, 2 of those with tubal-associated problems and additionally had ovulatory dysfunction, 3 with male-oriented issues, and 3 with both male-oriented issues along with ovulatory dysfunction in the development of tubal infertility. Sixty-four women with non-tubal infertility: 14 with male variable,16 with an ovulatory disorder, 18 with combined male factor in addition to ovulatory dysfunction, and 17 with idiopathic infertility the mean age of the women with tubal infertility was (34.2 ± 2.4 years) in comparison to those with different other causes of infertility (32 ± 5 years) as shown in Table 1.

An overall of 8 women had a history of pelvic inflammatory disease (PID), only three with tubal conditions. Consequently, 14 of 17 women with the tubal problem in our collection had no previous background of PID.

Table 2. Simple *C. trachomatis* antibodies analysis by MIF screening assay to predict tubal infertility

	Sample % age with positive MIF titer to *C. trachomatis* antibodies	Calculated values	MIF Sensitivity for *C. trachomatis* antibodies	MIF Specificity for *C. trachomatis* antibodies	Prediction value		Probability ratio	
					positive	negative	Favorable	unfavorable
Tubal VS non-tubal infertility	1. Tubal 12 of 18 (67%) 2. non-tubal 31 of 64 (48%)	P=0.3 OR=2.2 95% CI=0.71 to 8.01	65%	58%	29%	88%	1.15	0.71

P= power factor alpha, OR=odds ratio, CI=confidence interval, *C. trachomatus=Chlamydial trachomatis*

Table 3. CHSP60 antibodies measurement (nonspecifically) by ELISA in all the individuals with the tubal and non-tubal cause of infertility

	Sample % age positive to CHSP60 antibodies by ELISA	Calculated values	CHSP60 antibodies testing Sensitivity	CHSP60 antibodies testing specificity	Probability ratio
Tubal VS non tubal infertility	1.Tubal infertility 8 individuals from 18 (45%) 2.nontubal infertility 6 individuals from 64(9%)	P=0.004 OR=9.3 95% CI=2.1 to 43.2 Power =1.002 for n=0.05	44%	93%	6.1

P= power factor alpha, OR=odds ratio, CI=confidence interval, CHSP60=chlamydial heat shock proteins, ELISA=enzyme linked immunosorbent assay

Table 4. CHSP60 Antibodies measurement specifically in individuals with tubal infertility and with positive MIF titer in comparison with non-tubal infertility or negative MIF titer

	Sample % age positive to CHSP60 antibodies by ELISA	Calculated values	CHSP60 antibodies testing Sensitivity for *C. trachomatis*	CHSP60 antibodies testing specificity for *C. trachomatis*	Prediction value		Probability ratio
					positive	negative	
Tubal VS non tubal infertility	1. tubal 8 of 12 (67%) 2. non-tubal 7 of 70 (10%)	P=0.0000412 OR=31.02 95% CI=7 to 220	70%	93%	58%	86%	10

P= power factor alpha, OR=odds ratio, CI=confidence interval, *C. trachomatus=Chlamydial trachomatis*, CHSP60=chlamydial heat shock proteins

Thirteen individuals were located to have endometriosis at the time of laparoscopy, and 10 of these women without any ovaries or tubes related problem. Eight women had a prior background of ectopic pregnancy, and likewise, three of these females have normal tubes at laparoscopy after the resolution of the ectopic gestation (treated with methotrexate or direct salpingostomy). The remainder of our selected individuals diagnosed infertility with male issues or related to ovulatory aspects. A positive MIF titer in 12 of 18 (67%) women with the tubal issue and 31 of 64 (48%) with various other causes of infertility (P=0.3, OR = 2.2, 95% CI = 0.71 to 8.01). Hence, screening assessment for tubal conditions, MIF has a sensitivity of 65% and specificity of 58%, with a positive prediction of 29% and the negative prediction of 88%. Calculation of probability ratio revealed that MIF screening for *C. trachomatis* antibodies is not a helpful medical test for the prediction of the tubal problem in the individual with infertility (favorable possibility proportion = 1.51 as well as unfavorable chance ratio = 0.7), as shown in table 2.

The CHSP60 antibodies found in 8 of 18 (45%) women with the tubal problem in addition to 6 of 64 (9%) women with other causes of infertility, power factor alpha α P.=0.004, OR = 9.3, 95% CI = 2.1 to 43.2, probability ratio=6.1, power = 1.002 for n = 0.05). Consequently, as an indicator of tubal infertility, CHSP60 antibody testing has a sensitivity level of 44% and specificity of 93%, as shown in table 3.

For diagnosis of *Chlamydial* linked tubal problems (specified as the tubal condition with a positive MIF titer), the CHSP60 test performed in 8 of 12 (67%) patients with tubal disease in addition to 7 of 70(10%) females with other causes of infertility or with a negative MIF titer (P.=0.0000412, OR =31.02%, CI = 7 to 220). Therefore, the level of sensitivity of the CHSP60 assay for discovering *C. trachomatis* linked tubal disease is 70%, along with its 93% specificity. The positive value of prediction for the CHSP60 assessment for the medical diagnosis of tubal infertility is 58% as well as the negative value of prediction is 86%, as shown in Table 4. A person with the tubal condition is 6.1 times more likely than women without

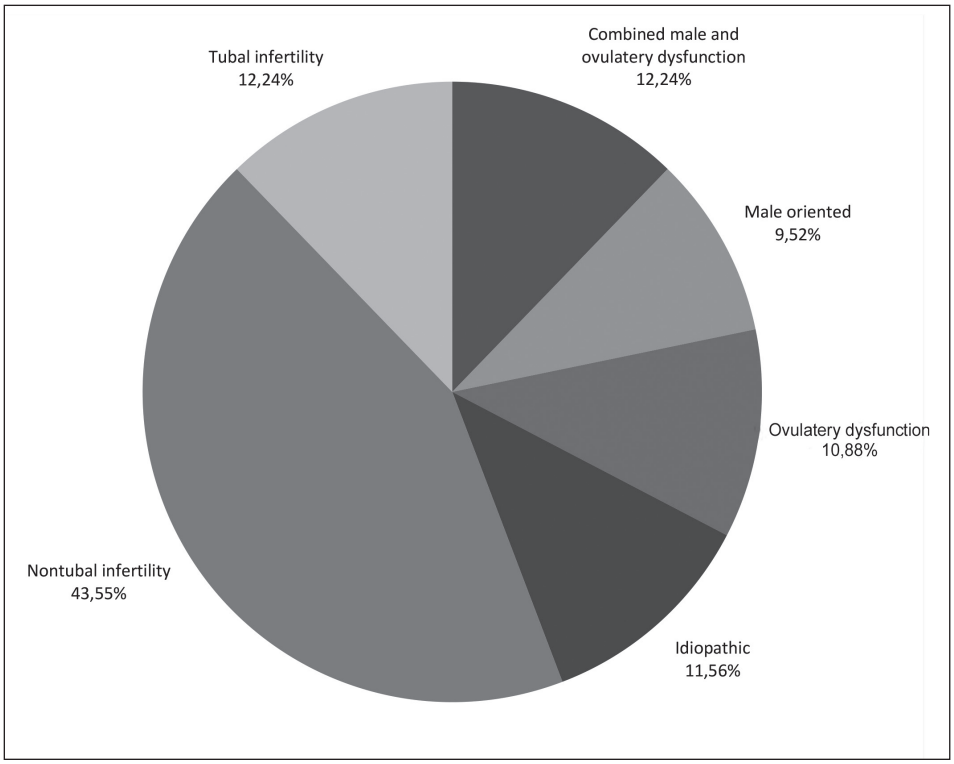

Fig. 1. Causes of infertility in a sample of the population.

tubal problems to have antibodies to CHSP60 (positive probability proportion).

The structure of tubal and non-tubal causes of infertility in the population sample selected for this study is presented in Figure 1.

DISCUSSION

CLINICAL FINDINGS

The determination of CHSP60 antibodies was a more accurate and particular technique to identify tubal infertility associated with c. trachomatis infection. The MIF was a more sensitive but less specific diagnostic method.

It has been recommended that C trachomatis anti- body screening might be valuable as a testing parameter in diagnosis for tubes related infertility [32]. As a forecaster of tubal condition, we located that the MIF for *C. trachomatis* test has limited specificity of merely 58% and a level of sensitivity of 65% for anticipating tubal condition in our center population.

The negative and positive ratios of 1.51 and 0.71, respectively, indicate the low sensitivity of MIF for diagnosis of tubes associated problems. The seroprevalence of *C. trachomatis* in most grown-up populations is 40% to 60% [33]. The MIF assessment permits a useful and more specific discovery of antibodies against different chlamydial strains [34]. Furthermore, Dabekausen et al.'s [5] interpretation of tubal disease consisted of tubo-peritoneal adhesion in addition to independent tubal pathology seen at laparoscopy. In contrast, in our collection, we specified tubal condition as the direct exposure of bilateral distal tubal clog or considerable peri tubal adhesion.

The degree of sensitivity of this evaluation for finding tubal condition is negative; the 92% specificity of this test for finding tubal condition makes it an important assay. The favorable probability proportion of CHSP60 antibodies screening found out the presence of the tubal condition in an unselected population of individuals providing for infertility was 6.1, making it an outstanding examination for predicting tubal presence associated infertility. The CHSP60 antibodies screening will help reveal the tubal damages resulting from *C. trachomatis* infections and help differentiate with other infertility causes.

Brunham et al. [23] previously reported that 19 of 21 (91%) females with ectopic pregnancy along with seropositive for *C. trachomatis* had antibodies to CHSP60. Three of these four women had CHSP60 antibodies (75%) Just 4 of these seven females had an apparent tubal problem.

STRENGTHS AND LIMITATIONS

This study's strengths are selecting individuals, especially those with the infertility issue associated with tubal factors and full patient background (e.g., age, BMI, ethnic background, essential language, gravidity and parity, other comorbidities, previous infections, present medications). This research study is a suitable rep evaluation for tubal infertility risks evaluation in the Kyiv, Ukraine population and also contributes to literary works on the prospective impact on the female with infertility connected with fallopian tubes.

Limitations of the study included the prospective case-control nature of the study, restricting the data collection. The sample size was also very limited as only those individuals were included whose infertility problem was explicitly associated with fallopian tubes.

The data was collected only from three institutes. There are regularly institutional propensities in providing medical diagnosis and treatment to women, and duplicating this research study with multiple research studies would undoubtedly reinforce our results' generalizability.

Women with tubal infertility, age less than 40 years or more than 25 years, with ovulatory dysfunction, endometriosis, ectopic pregnancy, and women with routine gynecological check-up were included.

Females with a previous history of autoimmunity, oncology, or hypersensitivity were excluded.

RESEARCH IMPLICATION

It is not well understood that CHSP60 antibodies in serum itself a reason for tubal infertility, or it sets off the activation of a few other chemical cascades in the body that results in this pathology's growth. Further immunochemical studies need to be done on this parameter. Given the exploratory nature of the results, we await verification of our research from future research studies. We also hope to discover associations amongst tubal infertility and various other kinds of heat shock proteins (HSP) i. e HSP 10, 70, 90, 110, and the decision of specific criterion to diagnose the linked etiology.

CLINICAL IMPLICATION

Among the essential factors for screening for chlamydia, unlike other sexually transmitted disorders as a cause of the tubal problem, interestingly infertility is the significant outcome of genital chlamydia infections in women, and mostly these infections are asymptomatic. Non-treated and ignored infections can spread into the upper genital system and likewise trigger PID (pelvic inflammatory disease) with resultant ectopic gestation as well as tubal occlusion and other complications. Numerous study studies have shown that most women with the tubal cause of infertility did not mind a history of chlamydia infection [4]. These examinations would undoubtedly be a fast and definite helpful approach for establishing the diagnose in these women without various other unwanted and expensive interventive treatment and diagnostic measures.

OUTCOME

The performance of the CHSP60 antibodies assessment depends upon its high specificity (92%) and good positive ratio (6.1) for the presence of tubal infertility. The negative MIF analysis has an excellent (85%) negative value of prediction that makes it useful alone in diagnosing tube-related infertility.

A positive MIF assay combined with CHSP60 antibody screening generates an extraordinary *C. trachomatis*-linked tubal infertility (good possibility ratio= 10). The CHSP60 antibodies evaluation and MIF testing should exist as an analysis tool as part of the first infertility examination. The women with a positive CHSP60 antibody assay might consider moving into IVF-ET treatment instead of finding extra interceptive and expensive screening examination, mainly IVF-ET seems specifically reliable in treating tubal-associated infertile females that have antibodies to the CHSP60 [34].

CONCLUSIONS

The *Chlamydia Trachomatis* infection is one of the significant risk elements for the development of tubal infertility. The measurement of CHSP60 antibodies by ELISA in serum samples is a particular extra parameter to detect tubal infertility related to chlamydial trachomatis infection instead of primary antibody *C. Trachomatus* as well as using a less specific microimmunofluorescence (MIF) strategy. The use of both methods together at the preliminary diagnostic evaluation of women may help provide the prompt diagnosis as MIF has a lot more sensitivity and CHSP60 antibodies by ELISA is extra particularly specific for medical diagnosis of *Chlamydia Trachomatis* linked tubal infertility.

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ORIGINAL ARTICLE

HYPODYNAMIA AS A FACTOR MODIFYING FUNCTIONAL MORPHOLOGY OF HUMAN PLACENTA

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ABSTRACT

The aim: Study of the functional morphology of placenta in a sedentary lifestyle of a woman during pregnancy.

Materials and methods: Object of the study: placentas obtained as a result of deliveries at term from women, urban residents, aged 20–40 years old, leading a sedentary lifestyle, and patients with a sufficiently high level of physical activity, the criteria of which corresponded to WHO recommendations. Immunohistochemical and morphometric studies of the placentas were carried out, followed by statistical analysis

Results: Prerequisites for reducing the efficacy of the functioning of fetoplacental complex with a sedentary lifestyle were sclerosis, the formation of intervillous fibrinoid and fibrinoid substitution of terminal villi. The inclusion of compensatory mechanisms in the form of placental hypertrophy, angiomatosis, sinusoidal transformation of the capillaries of terminal villi, thinning of the syncytiocapillary membrane associated with an increase in the content of von Willebrand factor in the villus syncytiotrophoblast, in aggregate, normalizes the exchange between maternal and fetal blood and creates certain prerequisites for the successful completion of pregnancy. However, thinning of the syncytiocapillary membrane increases the risk of rupture and direct contact of the internal media of the mother and the fetus.

Conclusions: Sedentary lifestyle of a pregnant woman leads to structural and functional changes in the placenta, which can be a serious prerequisite for the development of pathological abnormalities in the function of the "mother-placenta-fetus" system. To a certain extent, these changes are leveled due to compensatory processes in the placenta, the margin of efficacy of which needs further investigation.

KEY WORDS: placenta, sedentary lifestyle, pregnancy

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INTRODUCTION

The negative effect of physical inactivity on human health is a well-known and scientifically proven fact. A sedentary lifestyle and hypodynamia have become a kind of "portrait" of the inhabitants of the cities of Europe and, in particular, Ukraine. Among practicing obstetricians-gynecologists, there is still a conviction about the benefits of reducing physical activity during pregnancy, which supposedly reduces the risk of its premature termination. At the same time, scientific studies of recent years have proven the unambiguously negative effect of physical inactivity on general reproductive health and the course of the gestational process, condition of the fetus and newborn [1, 2, 3]. Experimental data on limiting the motor activity of pregnant animals are very indicative. In every fifth case, there was a resorption of the fetus and stillbirth, and in 35% of cases the offspring were found to be non-viable [4]. A prerequisite for pathological abnormalities during pregnancy, delivery and the postpartum period was endometrial hypoplasticity, combined with a decrease in contractile abilities of the myometrium [5]. Based on this, it is logical to assume the existence of deviations in the morphofunctional state of the placenta, the main provisional organ that plays a decisive role in the effective functioning of the mother-placenta-fetus system.

THE AIM

The aim of the was to study of the functional morphology of placenta in a sedentary lifestyle of a woman during pregnancy.

MATERIALS AND METHODS

Twenty placentas obtained as a result of deliveries at term were studied. Inclusion criteria: age 20–40 years; permanent residence in the city; the absence of clinically significant risk factors for obstetric pathology and concomitant somatic pathology requiring follow-up and treatment by appropriate specialists; no professional sports activities.

According to the WHO recommendations, physical activity of medium intensity, which includes walking at a medium or fast pace, for at least 150 minutes per week (about 20 minutes per day) should be considered normal

Table 1. Gestational age, mass-height and placentometric indicators in comparison groups

Indicators	"HD" group ($\overline{X} \pm S\overline{x}$)	"C" group ($\overline{X} \pm S\overline{x}$)
Gestation period, weeks	38.7 ± 0.4	39.8 ± 0.3
Average weight of placenta, g	633.3 ± 55.8	620.8 ± 44.0
Placental-fetal index	0.185 ± 0.011	0.173 ± 0.061
Weight of newborns, kg	3.50 ± 0.17	3.51 ± 0.15
Height of newborns, m	0.53 ± 0.01	0.53 ± 0.01
Height-weight index, kg/m^2	12.16 ± 0.73	12.28 ± 0.52

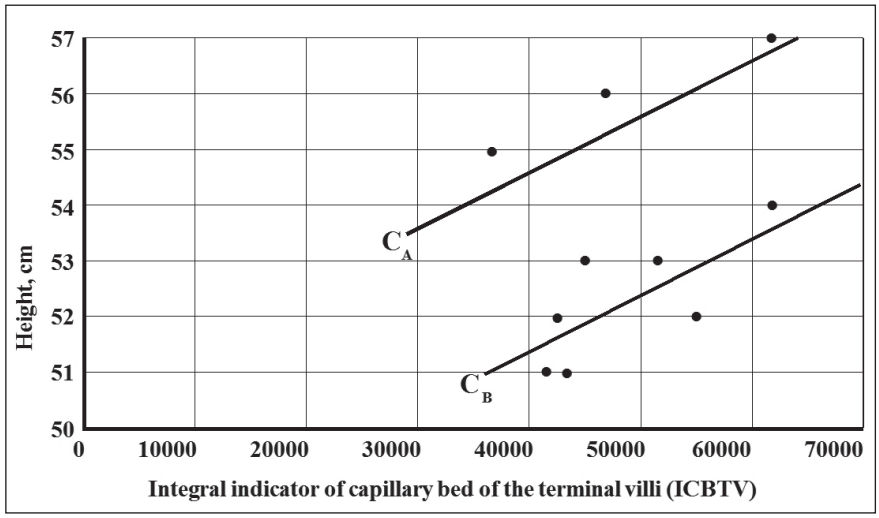

Fig. 1. Relation between the height of newborns and the integral indicator of the capillary bed of the terminal villi of placenta in the control group.

physical activity for this age group. In this case, the training session should be divided into time periods lasting at least 10 minutes [6, 7].

Women included in the study were offered a questionnaire that included 28 questions of self-assessment of the level of physical activity in everyday life using the multiple choice answer scheme. An objective assessment of the degree of motor activity of women was carried out by comparing the results in the questionnaire and the data of the OMRON Walking Style One 2.0 pedometer (Japan) that every woman wore for 10 days, followed by calculating the average distance (number of steps) traveled per day and duration of episodes of continuous physical exercise.

As a result, all cases were divided into the following observation groups:

– "HD" ("hypodynamia") – patients with a low level of physical activity, the total duration of episodes of physical activity (walking) for which did not exceed 10 minutes per day, and the distance traveled was less than 2000 steps per day (10 individuals);

– "C" – control group composed of women with a fairly high level of physical activity, the criteria of which were consistent with WHO recommendations (10 individuals).

The gestational age at the time of labor, the weight, height and height-weight index of the newborns (Quetelet index), the weight and size of the placenta, as well as the placental-fetal index were taken into account.

The placenta tissue obtained in the middle part of its radius was fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin-eosin, picro-fuchsin according to Van Gieson, gallocyanin-chrome alum according to Einarson for total nucleic acids, and periodic acid Schiff reaction was performed.

To determine the von Willebrand factor in the cytoplasm of endotheliocytes and syncytiotrophoblast, an immunohistochemical study was performed using antibodies from Prime-BioMed (Russian Federation). Morphometric studies were carried out on computer images of placental micropreparations using an Axiostar-plus microscope from Zeiss (Germany) with a Progress-C10+ camera using Video Test-3 software (Russian Federation). The number of terminal villi and the total number of capillaries in them within the photograph area were calculated, the thickness of the syncytiocapillary membranes and the optical density of the von Willebrand-positive cytoplasm of the syncytiotrophoblast of the villi were measured.

Statistical analysis of normally distributed data was carried out by parametric methods. Statistical hypotheses were tested using the t factor. Conclusions regarding statistical hypotheses were generated at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The average gestational age at the end of pregnancy was slightly longer in the control group than in the "HD" group, despite the fact that delivery was at term in all cases. At

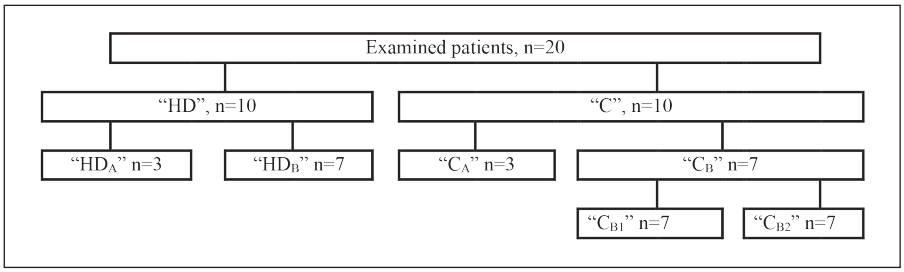

Fig. 2. Overall study design.

the same time, placentometric and weight-height indices did not significantly differ between the observation groups (Table 1).

The absence of a significant difference in the indicators we have obtained may be due to the fact that under the actual conditions of clinical observation, in addition to hypodynamia, there are many other external and internal factors that affect the placenta, which can not be taken into account, which may include diseases incurred during pregnancy, nutritional peculiarities, environmental hazards, etc. At the same time, favorable perinatal outcomes, despite the impact of alterative factors, are determined by the compensatory resources of placenta.

The results of numerous studies indicate an inevitable disturbance of blood supply to peripheral tissues associated with hypodynamia under both clinical and experimental conditions, based on which one should expect the presence of such disorders in the placenta [8, 9, 10]. To assess the degree of development of the capillary bed of terminal villi, we used an integral indicator, calculated as the product of the average number of capillaries of the terminal villi in a still frame (x100) and the mass of placenta: "Indicator of capillary bed of the terminal villi" (ICBTV). [11]. Comparison of the height of newborns and ICBTV made it possible to distinguish two subgroups in the "C" group: "C_A" and "C_B", in which a positive relation between a newborn's height and the placenta ICBTV value was observed, but at a different level (Fig. 1).

In the "C_A" subgroup, the newborns were taller (55–57 cm), the placentas turned out to be larger, and the placental tissue was mature histologically, there was stromal sclerosis of both large and terminal villi, intervillous fibrinoid was often present. The course of the gestational period in these women was complicated by anemia of the first degree. Placental hypertrophy with the phenomena of capillary hyperplasia of terminal villi (angiomatosis) concomitant with sclerotic processes and the formation of intervillous fibrinoid should obviously be considered as manifestations of adaptive processes associated with the indicated concomitant pathology, which in turn led to some acceleration of fetal development.

In the "C" group, the "C_B" subgroup was also distinguished (see Fig. 1), in which, on the basis of the results of evaluating the weight-height indicators and calculating the ICBTV values of placentas, two subgroups were distinguished: "C_{B-1}" and "C_{B-2} "(Fig. 2). The gestational process in women of the C_{B-1} subgroup proceeded without any complications. The newborns had a height of 52–54 cm, the weight of the placenta was about 600 g. Histological signs of placental damage in the form of sclerosis of the stroma of the villi, the formation of fibrinoid in the intervillous space were minimally presented, which allowed to consider these cases as "pure" control with physiological course of pregnancy (Fig. 3).

In the "C_{B-2}" subgroup, the course of pregnancy was complicated by mild anemia; newborns had a height of about 51 cm, the placenta weight was in the range of 450-550 g. Histologically, pronounced sclerosis of the villi and accumulation of fibrinoid took place. In these cases, normalization of the placenta capillarization occurred in different ways: due to placental hypertrophy, due to hyperplasia and an increase in the density of terminal villi, or due to their angiomatosis. However, of all the cases presented in the control group, the body length of the newborns in this subgroup turned out to be the smallest, which reflects the minimum severity of proliferative processes in them.

In all placentas of women with a low-active lifestyle ("HD" group), indisputable signs of damage to the placental tissue were revealed histologically: large and numerous intervillous foci of fibrinoid, many terminal villi replaced by fibrinoid; severe pericapillary sclerosis in functioning villi (Fig. 4).

An analysis of the relationship between the body length of newborns and placenta ICBTV allowed us to distinguish two subgroups in the "HD" group: "HD_A" and "HD_B" (see Fig. 3; Fig. 5).

In the placentas of "HD_A" subgroup, which had the largest mass (about 800 g), concomitant with pronounced sclerosis of placental villi and accumulation of fibrinoid, ICBTV varied within normal limits, and newborns had maximum height (55-60 cm), forming a positive relationship between ICBTV and newborn height. In "HD_B" subgroup, a similar relationship was found at a lower level: the placenta mass was 500–700 g with the height of newborns 50–54 cm. Histologically, there was even more pronounced sclerosis of terminal villi and accumulation of fibrinoid. In only one case, the placenta weight was minimal with the absence of angiomatosis of terminal villi, which apparently directly affected the severity of proliferative processes in the fetal tissues, and therefore its height (47 cm).

Thus, we can assume that the effect of alterative factors insignificant in intensity ("HD_A") leads to the development of compensatory processes in the placental tissue in the form of hypertrophy, hyperplasia, and angiomatosis of terminal villi, which is clinically manifested in intrauter-

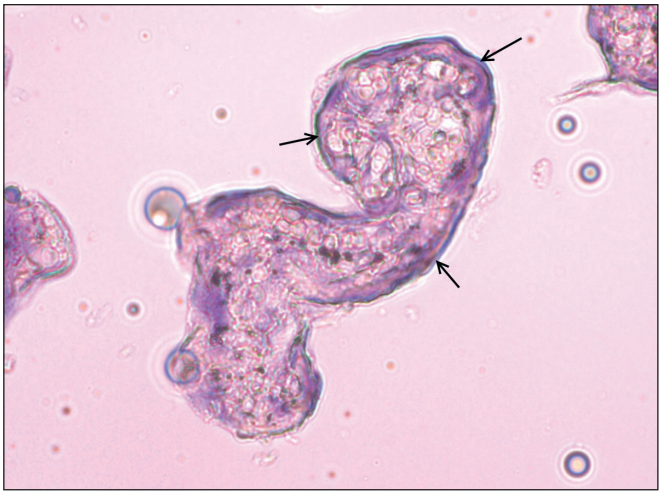

Fig. 3. Placental villus of a woman from "C" group. Antigen-positive linear sites in syncytia. Immunohistochemical reaction to von Willebrand factor. Magnification x1000.

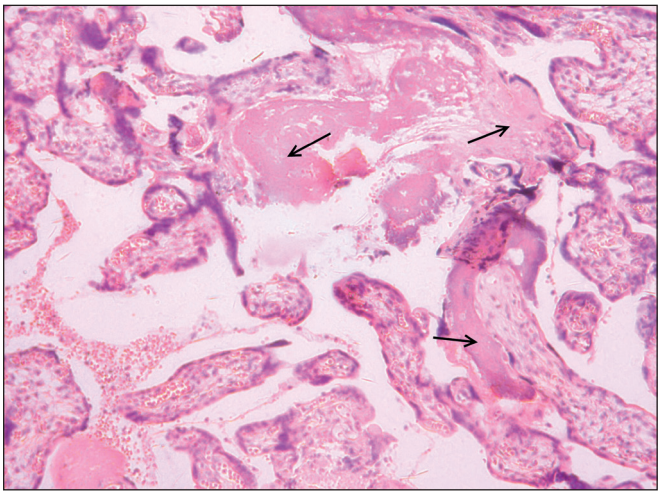

Fig. 4. Placenta of a woman from "HD" group. Space-occupying masses of fibrinoid are seen in the intervillous space. Hematoxylin and eosin staining. Magnification x100.

ine acceleration (tallness) of newborns. Large reserves of the compensatory potential of placenta when exposed to several or more intense damaging factors are confirmed by the constant value of ICBTV, despite the greater severity of signs of "aging", sclerosis in the placenta ("HD_B").

The average height of newborns, at the same time, corresponds to that in the physiological course of pregnancy (control), although it is much less than in the subgroup "HD_A". It is logical to assume that in order to compensate for the expressed alternative effects that lead to sclerosis of the stroma in the villi secondary to a sedentary lifestyle of a pregnant woman, a certain additional compensatory mechanism should be initiated in the placenta, which allows stabilizing the function of the fetal-placental complex.

As is known, the exchange between the mother and the fetus in the last weeks of gestation occurs in a diffuse manner through syncytiocapillary membranes forming in the terminal villi of the placenta in the process of shifting the syncytium nuclei to one focus with the formation of the syncytial nodule. The syncytiocapillary membrane consists of the following layers: syncytium cytoplasm, syncytium basal membrane, villus stroma, capillary basal membrane with fetal blood, capillary endothelium. At the end of gestation period, the thickness of the syncytiocapillary membrane reaches 3-5 microns. [12]. Obviously, thinning of the syncytiocapillary membrane leads to an intensification of exchange between the mother and the fetus in the placenta.

We measured the syncytiocapillary membrane thickness. It turned out that the thickness of this most important structural part of the placenta of women in the control group was significantly less than in the case of a sedentary lifestyle during the gestation period (Table 2). The thickness range of the syncytiocapillary membrane in the placenta of the control group was 2.07–4.92 μm, and the same in "HD" group was 1.13–3.59 μm. In the latter case, terminal villi in which the capillary protrudes beyond the rounded contour of its cross section are often encountered. In addition, syncytial nodules are generally larger than in the control, often with karyopicotic nuclei.

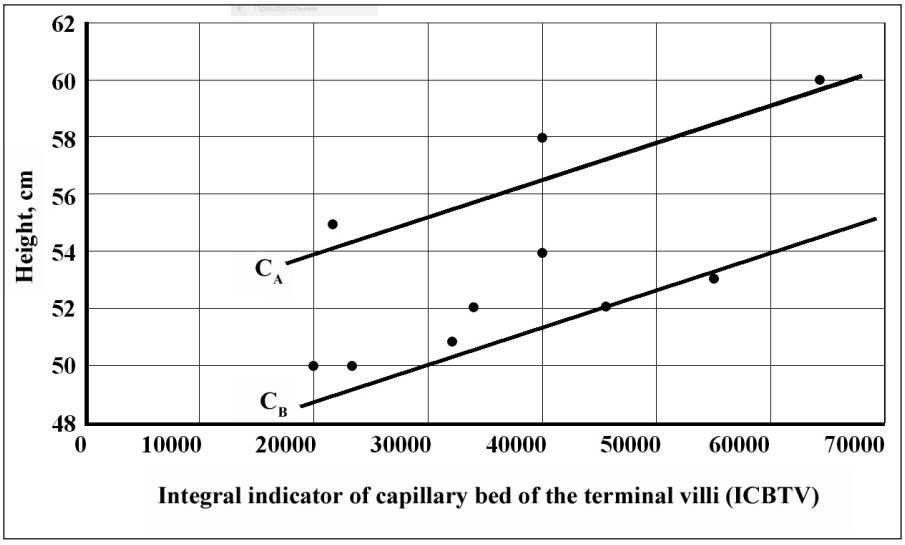

Fig. 5. The relationship between the integral indicator of the capillary bed of terminal villi of the placenta and the height of newborns in the "HD" group.

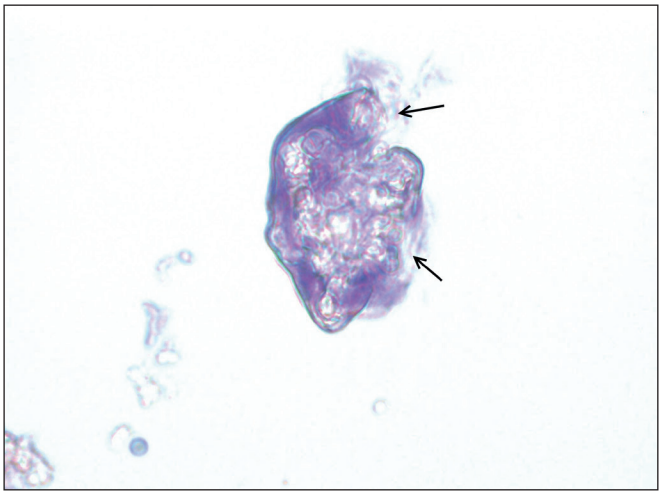

Fig. 6. A placental villus of a woman from "HD" group. There are two sites of destruction of the syncytiocapillary membrane with open mutual contact of the fetal and maternal blood. Immunohistochemical reaction of von Willebrand factor. Magnification x1000.

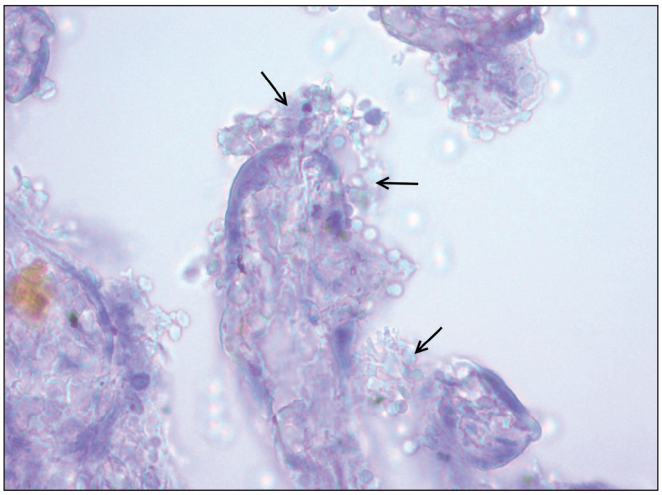

Fig. 7. A placental villus of a woman from "HD" group. Syncytium is lost on a large portion of the surface of the villus. The latter is blocked by fresh blood clots. Immunohistochemical reaction of von Willebrand factor. Magnification x1000.

The fact that the syncytiocapillary membranes are thinning can explain the nature of the ruptures of the syncytiocapillary membrane that are often observed in the placentas of "HD" group with the formation of a pattern of fetal blood "explosion" (Fig. 6). Obviously, this is a consequence of the compensatory thinning of the syncytiocapillary membrane, which occurs to normalize the exchange between the mother and the fetus in the placenta, often leading to the above-described acceleration of fetal growth.

The results of our immunohistochemical study of the placenta using antibodies to von Willebrand factor are noteworthy. This blood plasma glycoprotein, which is formed in the Weibel-Palade bodies of endothelium cells, assures platelet attachment to the damaged vessel by binding to other proteins, primarily coagulation factor VIII [13]. It was found that the endothelium of the capillaries of the placental terminal villi is practically not preserved, which is apparently due to the development of severe hypoxia during transection of the umbilical cord and separation of the placenta from the uterus. However, in the vessels of large villi, partial preservation of the endothelium with aggregation of erythrocytes on the basal membrane in the areas of its absence takes place. Synthesis of von Willebrand factor occurs not only in the cytoplasm of endotheliocytes, but also in the syncytial cover of the villi, which plays a huge role in preventing the mixing of maternal and fetal blood. When s syncytium site on the surface of a villus dies, fibrin begins to be deposited immediately (under the influence of von Willebrand factor), blocking the syncytiocapillary membrane. Antigen-positive substances are located linearly in the outer layer of syncytiocapillary membranes (Fig. 7).

Measurement of optical density of antigen-positive, i.e. labeled portions of the syncytiocapillary membranes revealed its increase in placentas of the "HD" group versus the control (see Table 2). An increase in the content of von Willebrand factor in the thinned syncytiocapillary membranes of the terminal villi of the placenta can be regarded as an adaptation mechanism that prevents direct contact of the internal media of the mother and the fetus.

CONCLUSIONS

1. In placentas of women with a sedentary lifestyle, there is an increase in the degree of formation of intervillous fibrinoid, fibrinoid substitution, and villous sclerosis, which leads to the "shutdown" of villi and, consequently, to a decrease in the normal functioning of the fetoplacental complex. Differences in mean somatometric

Table 2. The thickness of the syncytiocapillary membranes of the terminal villi and the optical density of the antigen-positive sites of syncytium during immunohistochemical reaction of von Willebrand factor

Group	Thickness of the syncytiocapillary membranes of the terminal villi, μm ($\overline{X} \pm S\overline{x}$)	Optical density of the antigen-positive sites of syncytium (conventional units of optical density) ($\overline{X} \pm S\overline{x}$)
C	3.55 ± 0.16	0.153 ± 0.009
HD	2.4 ± 0.12**	0.198 ± 0.004*

Remarks:
* – p<0.01 versus the control;
** – p<0.001 versus the control.

indices of newborns in comparison groups, however, are not statistically significant.
2. Hypertrophy of placenta with an increase in its weight and volume, villus hyperplasia, terminal villi angiomatosis, sinusoidal transformation of terminal villi capillaries reflect the huge compensatory potential of the placenta, and, according to morphometric studies, these compensatory mechanisms are equally used in both groups studied.
3. Thinning of the syncytiocapillary membrane was revealed in terminal villi of the placenta of women with a sedentary lifestyle, associated with an increase in the content of von Willebrand factor in the syncytiotrophoblast of the villi, which should be interpreted as triggering of another compensatory mechanism that normalizes the exchange between maternal and fetal blood with concomitant increased risk of syncytiocapillary membrane rupture and direct contact of the internal media of the mother and fetus.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

VAGINAL CUFF INFECTION AFTER HYSTERECTOMY IN UKRAINE

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ABSTRACT

The aim: To obtain the first estimates of the current prevalence of vaginal cuff infection after hysterectomy and antimicrobial resistance of causing pathogens in Ukraine.

Materials and methods: We performed a retrospective multicenter cohort study was based on surveillance data. The study population consisted of women who had an abdominal, vaginal or laparoscopic hysterectomy from 2017 to 2019 in 7 women hospitals of Ukraine. Definitions of vaginal cuff infections were used from the Centers for Disease Control and Prevention's National Healthcare Safety Network, USA.

Results: Total 12.6% women's after hysterectomy had vaginal cuff infections. Of these cases, 20.3% after abdominal, 15.5% vaginal and 4.1% laparoscopic hysterectomy were identified. The predominant pathogens of VCUF infections were: *Escherichia coli* (18.6%), *Enterobacter* spp. (12.4%), *Staphylococcus aureus* (10.8%), *Streptococcus* spp. (9,7%), *Klebsiella pneumoniae* (8.2%), *Pseudomonas aeruginosa* (7.6%), *Enterococcus faecalis* (7,0%) and *Proteus* spp. (7.0%).

Methicillin-resistance was observed in 12.9% of S. *aureus* (MRSA) and 9.7% CoNS. Carbapenem resistance was identified in 7.3% of *P.aeruginosa* isolates. Resistance to third-generation cephalosporins was observed in 8.9% *K. pneumoniae* and *E.coli* 11.9% isolates. The overall proportion of extended spectrum beta-lactamases (ESBL) production among *Enterobacteriaceae* was 22.7%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (32.6%, vs 12.3%).

Conclusions: Vaginal cuff infections in women after hysterectomy are common in Ukraine and most of these infections caused by antibiotic-resistant bacteria. The incidence of VCUF infections after hysterectomy differs depending on the type of surgical procedure.

KEY WORDS: Hysterectomy, abdominal, vaginal, laparoscopic, vaginal cuff infection, pathogens, antimicrobial resistance

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INTRODUCTION

Hysterectomy is one of the most commonly performed gynecological surgeries, which can be carried out via a vaginal, abdominal or a laparoscopic approach for treatment adenomyosis, uterine prolapse, cervix, or ovaries and other diseases. Vaginal cuff (VCUF) infection remains a common complication after hysterectomy surgery in Ukraine and results in significant patient morbidity. However, studies of prevalence of VCUF – infection are scant.

According to literature, gynecologic surgical procedures pose a potential risk transmission of pathogenic microorganisms from the skin or vagina and endocervix may migrate to operative sites and can result in VCUF – infections [1]. Estimated, prior to the advent of routine antimicrobial prophylaxis, pelvic infection rates after hysterectomy were high as 33% [2]. Therefore, guidelines of many countries, including Ukraine, recommend the use of antibiotic prophylaxis for gynecological surgery. However, the effect of antibiotic prophylaxis for VCUF – infections after hysterectomy is not fully understood. The majority of patients typically present after hospital discharge with moderate purulent vaginal discharge [1].

Current guidelines for the treatment of infections recommend the immediate prescription of antimicrobial medicines as soon as the infection is diagnosed. Broad spectrum antimicrobials should be prescribed even before the culture results are known in order to cure the most probable infection agents [3-8]. However, the literature data on the etiology and resistance of pathogens caused Reproductive Tract Infections varies considerably [9, 10]. Inadequate therapy extends the duration of hospitalization and provokes a need for additional courses of antimicrobial therapy that makes treatment more expensive.

The epidemiologies of VCUF – infections after hysterectomy in Ukraine are not studied. The previous reports of Reproductive Tract Infections in Ukraine have been limited only to Endometritis [11] and Episiotomy infections [12].

THE AIM

The aim of this study was to obtain the current prevalence of VCUF – infections after hysterectomy and antimicrobial resistance of causing pathogens in Ukraine.

MATERIALS AND METHODS

STUDY DESIGN AND POPULATION

We performed a retrospective multicenter cohort study was based on surveillance data of Reproductive Tract Infections in women's. Our study included patients undergoing an elective abdominal, vaginal or laparoscopic hysterectomy for benign reasons in 7 women hospitals of Ukraine from January 1st, 2017 to December 31st, 2019 (Table I). In the current study, we included 1491 women's (less than 50 years of age or if over 50 still menstruating) who were local residents. However, 236 of these were excluded from this study. Exclusion criteria were preoperative antibiotic use within one month prior to surgery, surgery for malignant disease and operations that for other reasons required preoperative antibiotics. 43 patients were excluded for missing or inadequate vaginal smears, mostly due to interfering menstrual blood.

DEFINITION AND DATA COLLECTION

In our study the CDC/NHSN (Centers for Disease Control and Prevention/National Healthcare Safety Network) definition of VCUF – infections was used [13]. In study period were analyzed the inpatient data and ambulatory medical records to identify VCUF – infections. We collected the data using structured NHSN Reproductive Tract Infection (REPR) Checklist for VCUF – infections. Full-text ambulatory medical records and relevant hospital records were reviewed for the all women's. Additional data form was created to extract microbiology (isolated pathogens and antibiograms) from inpatient data and ambulatory medical records. The follow-up of each patient was during 1 month days after hysterectomy. VCUF – infections were classified as superficial incisional SSI as per CDC/NHSN criteria [13].

MICROBIOLOGICAL SAMPLING AND SUSCEPTIBILITY TESTING

A purulent sample were collected with sterile swab on stick from the vaginal cuff from women with clinical symptoms of VCUF – infections and transported to microbiology laboratory. Results were not considered for more than two clinical isolates obtained from the same patient and the sample was considered to be contaminated. Microbial isolates from patients 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 according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In our study strains in the intermediate range were classified as resistant for data analysis.

ETHICS

The Shupyk National Medical Academy of Postgraduate Education (Kyiv, Ukraine) ethics committee approved the

Table I. Distribution of type hysterectomy in different women hospitals of Ukraine

Hospital	All patients No.	Type of hysterectomy		
		Abdominal No. of procedure	Vaginal No. of procedure	Laparascopic No. of procedure
A	174	25	69	80
B	180	43	78	59
C	185	38	79	68
D	175	41	80	54
E	184	49	79	56
F	176	43	74	59
G	181	37	83	61
Total	1255	276	542	437

Table II. Characteristics patients with VCUF infections after different type hysterectomy

Type of hysterectomy	All patients *n*	VCUF infections		95% CI*
		n	%	
Abdominal	276	56	20,3	17.9 – 22.7
Vaginal	542	84	15,5	14.0 – 17.0
Laparoscopic	437	18	4,1	3.2 – 5.1
Total	1255	158	12,6	11.7 – 13.5

Note

*CI-confidence interval

waiver of informed consent to participate in this study due to its retrospective design. All women's data were anonymised prior to the analysis.

STATISTICAL ANALYSIS

In our study prevalence of VCUF – infections was reported as the percentage of the total number of women who had been submitted to hysterectomy cases. We analyzed samples from women's in the context of a study about microbiology of VCUF infection and antimicrobial resistance of causing pathogens. The analysis of statistical data was performed using Excel (Microsoft Corp., Redmond, WA, USA). Results are expressed as median (range), mean standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. Comparisons were undertaken using Student's t-test and Fisher's exact test for categorical variables. Statistical significance was defined as $P<0.05$.

RESULTS

PREVALENCE OF VAGINAL CUFF INFECTIONS

During the study period (January 1st, 2017 and December 31st, 2019), of the 1,255 patients who underwent hysterectomy for benign indications, VCUF infections was diagnosed within 30 days in 158 (12.6%, 95% CI 11.7%, 13.5%, P<0.001). Incidence of VCUF infections after hysterectomy differed according to the surgery procedure types and women clinics. Of these cases, 20.3% (56/276) after abdominal, 15.5% (84/542) vaginal and 4.1% (18/437) laparoscopic hysterectomy were identified. Characteristics of patients with VCUF infections after different type hysterectomy are presented in Table II.

Prevalence of VCUF – infections was lower among women with adenomyosis who underwent laparoscopic hysterectomy compared with those who underwent abdominal hysterectomy for benign indications. Of the total cases VCUF – infections, 89.2% (141/158) were detected after hospital discharge. 94.3% (149/158) of patients with VCUF – infections did not return to the hospital for evaluation or treatment. These patients continued their treatment outside the hospital in outpatients.

ANTIBIOTIC PROPHYLAXIS

Routine antibiotic prophylaxis at gynecological surgery is standard practice in Ukraine, consistent with surgical guidelines internationally. Prophylactic antibiotic administration is generally timed to establish a bactericidal concentration in serum and tissue prior to surgical incision. Of 1255 women's, 89.9% were prescribed combination ceftriaxone and metronidazole pre-operative period. Ceftriaxone and metronidazole was also prescribed for 77.8% (123/158) participants meeting criteria for VCUF infections. Another 22.2% (35/158) were prescribed cefazoline and metronidazole. Overall, in this study 89.9% participants had a chart-documented prescription for β-lactam antibiotic prophylaxis, including 77.8% of women's with VCUF infections.

MICROORGANISMS CAUSING VCUF INFECTIONS

In this study, a total of 158 samples from women with VCUF infections after hysterectomy were analyzed. Among the 158 analyzed samples, 8.2% (13/158) did not show any microbial growth. The remaining 91.8% (145/158) samples were positive for pathogens with colony count higher than 10^5 CFU/mL and were included in the current study analysis. In this study, 84.9% VCUF infections were polymicrobial. A total 474 strains of microorganisms were identified. Among this isolates gram-negative bacilli make up 67.7% (321/474) and 32.3% (153/474) gram-positive cocci. The predominant pathogens of VCUF infections were: *Escherichia coli* (18.6%), *Enterobacter* spp. (12.4%), *Staphylococcus aureus* (10.8%), *Streptococcus* spp. (9,7%), *Klebsiella pneumoniae* (8.2%), *Pseudomonas aeruginosa* (7.6%), *Enterococcus faecalis* (7,0%), and *Proteus* spp. (7.0%). They are closely followed by *Klebsiella oxytoca* (4.6%), *Serratia* spp. (3.8%), Coagulase-negative staphylococci (3.2%), *Citrobacter* spp. (3.2%), *Acinetibacter* spp. (2.3%) and *Enterococcus faecium* (1.7%). Structure of the microorganisms differed according to the hysterectomy types. The distribution of microorganisms causing infections after different types of hysterectomy in Ukraine is shown in Table III.

Antimicrobial resistance of CAUSING pathogens

The staphylococcal isolates displayed a remarkable resistance to penicillin (84.7%) and erythromycin (69%), although there were some differences depending on the species. Staphylococcal isolates showed susceptibility to most of the other antimicrobials tested. No strains resistant to linezolid, teicoplanin, vancomycin, tigecycline, and fusidic acid were found. Methicillin-resistance was observed in 12.9% of *S. aureus* (MRSA) and 9.7% CoNS.

Streptococcal isolates demonstrated a noteworthy resistance against erythromycin (66.3%) and benzylpenicillin (53.1%), followed by ampicillin (32.7%) and tigecycline (18.4%). Most of the isolates were sensitive to rifampicin (87.3%), clindamycin (89.5%), gentamycin (91.1%), cefuroxime (94.3%), tobramycin (98.3%), and linezolid (99.4%).

Regarding the genus *Enterococcus*, *E. faecalis* isolates were not sensitive to those antibiotics to which they are intrinsically resistant (cefuroxime, clindamycin, and trimethoprim-sulfamethoxazole) and 78.7% of them were resistant to erythromycin. Approximately, 20% of the *E. faecalis* isolates displayed resistance to high levels of aminoglycosides (gentamycin, tobramycin) and around 8.2% was resistant to quinolones (ciprofloxacin and levofloxacin), and 4% to glycopeptides (vancomycin and teicoplanin).

The overall proportion of extended spectrum betalactamases (ESBL) production among Enterobacteriaceae was 22.7%. The prevalence of ESBL production among *E. coli*

Table III. Distribution of microorganisms, isolated from women with VCUF infections after different types of hysterectomy in Ukraine.

Microorganisms[(a)]	All isolates (n=474)	Type hysterectomy		
		Abdominal (No./% of isolates)	Vaginal (No./% of isolates)	Laparoscopic (No./% of isolates)
Gram-positive cocci	153	109 /7 1.2	31 /20.3	13 /8.5
Enterococcus faecalis	33	21 / 63.6	8 / 24.2	4 / 12.1
Enterococcus faecium	8	3 / 37.5	4 / 50.0	1 /12.5
Streptococcus spp.	46	23 / 50.0	17 / 37.0	6 / 13.0
CoNS[(b)]	15	13 / 86.7	0	2 / 13.3
Staphylococcus aureus	51	49 / 96.1	2 / 3.9	0
Gram-negative bacilli	321	176 / 54.8	127 / 39.6	18 / 5.6
Escherichia coli	88	62 / 70.5	23 / 26.1	3 / 3.4
Klebsiella pneumoniae	39	12 / 30.8	25 / 64.1	2 / 5.1
Klebsiella oxytoca	22	5 /2.7	13 / 59.1	4 / 18.2
Enterobacter spp.	59	28 / 47.5	28 / 47.5	3 / 5.1
Proteus spp.	33	20 / 60.6	13 / 39.4	0
Serratia spp.	18	11 / 61.1	6 / 33.3	1 / 5.6
Citrobacter spp.	15	6 / 40.0	9 / 60.0	0
Pseudomonas aeruginosa	36	27 / 75.0	7 / 19.4	2 / 5.6
Acinetobacter spp.	11	5 / 54.5	3 / 27.3	3 / 27.3

Notes:
(a) Used "The Bergey's Manual of Determinative Bacteriology" 9th Edition
(b) CoNS: Coagulase-negative staphylococci

isolates was significantly higher than in *K. pneumoniae* (32.6%, vs 12.3%, $p < 0.001$). *E. coli* was most sensitive (>90%) to ertapenem (100%), cefotaxime (99.1%), ceftazidime (99.4%), imipenem (98.9%), piperacillin/tazobactam (97.3%), and gentamycin (91.3%) but least susceptibility (<70%) was observed for moxifloxacin (54.2%), cefuroxime (61.8%), amoxicillin (65.2%), and levofloxacin (66.5%). Resistance to third-generation cephalosporins was observed in 11.9% *E.coli* isolates. No strains resistant to ertapenem were found.

Enterobacter spp. was most sensitive (>90%) to ciprofloxacin (97.3%), piperacillin/tazobactam (94.9%), cefotaxime (94.8%), ceftazidime (94.5%) and ticarcillin (91.5%). No strains resistant to cefepime, meropenem, imipenem, and ertapenem were found. *Enterobacter* spp. isolates ones exhibited a noticeable percentage of resistance against ampicillin/sulbactam (59.5%), ampicillin (52.1%), amoxicillin/clavulanic acid (51.4%), clindamycin (49.2%), ciprofloxacin (47.8%), gentamycin (43.5%), cefaperazon (41.3%) and ceftriaxon (31.5%).

K. pneumoniae isolates showed susceptibility to most of the other antimicrobials (meropenem, imipenem, levofloxacin, and gentamycin) tested, while these isolates ones exhibited a noticeable percentage of resistance against ampicillin (53.8%), amoxicillin/clavulanic acid (39.5%), ofloxacin (31.5%), and ciprofloxacin (28.8%). No strains resistant to piperacillin/tazobactam and ertapenem were found. Resistance to third generation cephalosporins was observed in 8.9% *K.pneumoniae* isolates. *Proteus* spp. was most sensitive (>90%) to imipenem (98.1%), gentamycin (97.5%), cefotaxime (93.6%), cefepime (91.5%), and ceftazidime (91.4%). No strains resistant to ertapenem piperacillin/tazobactam, and amikacin were found. In our study *P. aeruginosa* isolates demonstrated remarkable resistance to cefepime (47.1%), gentamycin (36.2%), and cefoperazone (33.5%), and was most sensitive to meropenem (97.2%), imipenem (88.6%), piperacillin/tazobactam (87.8%), ceftazidime (88.1%), amikacin (85.1%), ticarcillin (81.9%), ciprofloxacin (81.9%). No strains resistant to ertapenem were found. Carbapenem resistance was identified in 7.3% of *P.aeruginosa* isolates.

DISCUSSION

Results of our study have shown that cuff infection is an important problem for women after hysterectomy in Ukraine. During the study period (2016-2018) the prevalence of VCUF infection after hysterectomy was 12.6%. Of these cases, 20.3% after abdominal, 15.5% vaginal and 4.1% laparoscopic hysterectomy were identified. Hysterectomy is one of the most common major surgical procedures for women with benign gynecological diseases in Ukraine. This surgery may be done for different reasons, including adenomyosis, uterine prolapse and other problems. However, there are limited numbers of study that report VCUF – infection after hysterectomy.

The VCUF – infections were classified as superficial incisional SSI as per CDC/NHSN criteria [13]. In literature

reported Incidence rate of SSI after hysterectomy have ranged from 1.7 to 11% [14-19], while VCUF – infection ranged from 3.1 to 4.8%. [17-19]. In our study incidence rate of VCUF infections was lower among women with adenomyosis who underwent laparoscopic hysterectomy compared with those who underwent abdominal hysterectomy for benign indications. These findings are in parallel with previous reports that showed that minimally invasive hysterectomy is associated with more favorable perioperative outcomes and fewer postoperative complications, including SSI [20, 21].

Incidence rate of VCUF – infections vary depending on the whether or not post-discharge surveillance was used to identify infections. Reilly et al. [22] reported that the incidence of SSI after hysterectomy doubled when patients completed questionnaires after hospital discharge. In this study the CDC/NHSN criteria [13] were used for diagnosing VCUF – infections after hysterectomy. During the surveillance period VCUF infections was diagnosed within 30 days in 158 patients. Of these cases, 89.2% of VCUF infections were detected in post-discharge surveillance period.

Gynecologic procedures, including laparoscopy or laparotomy pose a potential risk transmission of pathogenic microorganisms from the skin or vagina and endocervix may migrate to surgical sites after hysterectomy and can result in VCUF – infections [1, 10, 23]. In vaginal surgery or hysterectomy, the endogenous flora of the genital tract the likely cause will be polymicrobial, consisting of anaerobes, Gram-negative aerobes and Gram-positive cocci [10]. In our study, 84.9% VCUF infections were polymicrobial and gram-negative bacilli make up 67.7%, and 32.3% gram-positive cocci from of all isolates. The predominant pathogens of VCUF infections were: *E. coli*, *Enterobacter* spp., *S. aureus*, *Streptococcus* spp., *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, and *Proteus* spp. Structure of the microorganisms differed according to the hysterectomy types (Table III).

Although current guidelines of many countries recommend the use of antibiotic prophylaxis for gynecological surgery, postoperative infections still occur [24, 25]. Clinical and therapeutic decisions are influenced by numerous factors, including antimicrobial resistance of the causative agents of VCUF infections. Optimally, the given antibiotic should be selected depending on the safety profile and local drug susceptibility. However, in Ukraine, there is no national network for the antimicrobial resistance surveillance. Our study showed that higher incidence rate of VCUF infections after hysterectomy in Ukraine was significantly associated with pathogens resistant to antibiotics. Antimicrobial resistance in the isolates associated with VCUF – infections showed, proportion of extended spectrum beta- lactamases (ESBL) production among *Enterobacteriaceae* was 22.7%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (32.6%, vs 12.3%). Among the gram-negative bacteria, third-generation cephalosporins resistance was found in 8.9% of *K. pneumoniae* and in 11.9% of *E.coli* isolates. Carbapenem resistance was reported in 7.3% of *P. aeruginosa* isolates. Methicillin resistance was reported in 12.9% of *S.aureus* isolates.

STRENGTHS AND LIMITATION

Our study is the first study reporting the prevalence of VCUF – infections in women after hysterectomy and antimicrobial resistance of causing pathogens in Ukraine. The absence of national surveillance data for VCUF – infections in Ukraine compelled us to rely entirely on data from the only existing study. The strengths of the study lie in the application of CDC/NHSN methodology. The CDC/NHSN criteria [13] were used for diagnosing VCUF – infections after hysterectomy. The follow-up of each patient was during 1 month days after hysterectomy. The screening of hospital and ambulatory records was a sensitive surveillance method for identifying VCUF – infections. The limitations of this study include its retrospective design and conduct at a in seven hospitals in Ukraine. Therefore, the results this study not be representative of other hospitals of Ukraine with different distributions of antimicrobial resistance of causing pathogens of VCUF – infections. However, this study provides valuable data as a first study for national surveillance of VCUF – infections and potential comparison with data from other countries.

CONCLUSIONS

The study showed that VCUF – infections after hysterectomy in Ukraine is a common occurrence and many cases are caused by pathogens that are resistant to antibiotics. Incidence of VCUF infections after hysterectomy differed according to the surgery procedure types. Minimally invasive (Laparoscopic) hysterectomy has lower infection rates than abdominal and vaginal hysterectomy. Given of the rapidly developing antimicrobial resistance, the policy of antibiotic use for prophylactic or treatment of VCUF infections in each region should be determined depending on local data on resistance to antimicrobials. Strategic planning and implementation of Reproductive tract infections in women surveillance is required.

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ORIGINAL ARTICLE

CLINICAL FEATURES OF GASTROINTESTINAL ULCERATIVE BLEEDING IN ELDERLY PATIENTS COMPLICATED BY CARDIO-VASCULAR PATHOLOGY

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ABSTRACT

The aim: To determine clinical and endoscopical features of gastroduodenal hemorrhages in elderly patients with concomitant cardio-vascular pathology in a way by studying, main indicators of the immune system for drawing up further tactics.

Material and methods: The study included 609 patients with ulcerative gastroduodenal bleeding, complicated by cardio-vascular system pathology in 2017-2019 years. The observed patients were distributed into the groups: I – patients, who received treatment according to the standard system of cardiovascular pathology treatment (n=541), II – "double" therapy (n=68). Control group consists of 20 relatively healthy patients were similar to the research group.

Results: Blood lost of a big amount and massive blood lost were noticed in 113 (18.56%±1.58) and 121 (19.87%±1.62) patients respectively. Active bleeding (F I) was revealed in 38 patients (6.24%±0.98), a high risk of hemorrhage relapse was determined in 486 patients (79.80%±1.63). Signs of recent hemorrhage were absent in 85 patients (13.96%±1.40). A high level of pro-inflammatory cytokines IL-6, TNF-α and a low activity of the anti-inflammatory mediator IL-10 define the process activity, their long-term circulation in patients with ulcerative hemorrhages of the gastro-intestinal tract are associated with unfavorable prognosis. In 5 cases conditionally-radical surgical interventions were performed. Palliative surgery – 3 patients ($p>0.05$).

Conclusions: The patients of second group ("double therapy") with big and massive blood loss was 2.7 times higher than similar indices in patients of the first group (standard therapy). The patients who received "double therapy" had 3.3 times more active hemorrhage percentage than the patients who received standard therapy ($p<0.05$).

KEY WORDS: ulcer bleeding, Forrest scale, cytokines

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INTRODUCTION

Gastroduodenal hemorrhages remain one of the most essential issues in urgent abdominal surgery. This group is presented by patients both with chronic pathology of the cardio-vascular system and acute myocardial infarction, arrhythmias with different origins, angina pectoris, conditions after coronary artery bypass grafting and stenting. For the last 3 years, there had been a growth in the number of acute ulcerative hemorrhages from the upper parts of the gastrointestinal tract I-IV degrees, especially in the elderly patients with concomitant cardiovascular pathologies [1]. Despite of using the modern minimally invasive methods (endoscopic hemostasis and hemorrhage relapse prevention), the mortality remains high and reach 10-20%, and post-operative mortality reaches 50% according to Fomin P. D., Shepetko E. M., Mennuni M.G., Halperin J. L., Kral J.B [2,3,4].

THE AIM

To determine the clinical features of gastroduodenal hemorrhages in elderly patients with concomitant cardio-vascular pathology in a way by studying basic clinical-endoscopic indicators, main indicators of the immune system for drawing up further tactics.

MATERIALS AND METHODS

Having studied and analyzed the dynamics of the course of gastrointestinal hemorrhages on the background of cardiovascular pathology including acute myocardial infarction, arrhythmias, anginas, conditions after coronary artery stenting, it has been revealed 609 elderly patients (according to the WHO classification – 61-90 years old). Among them males were 322 (52.8%), females – 287 (47.2%). Average age was 71.8 years old. The obtained data were distributed into the groups: I – patients, who received treatment according to the standard system of cardiovascular pathology treatment (n=541), II – "double" therapy (n=68). As the group of control, we selected 20 patients – relatively healthy patients (donors), who by age, gender, methodology of determining main indices were similar to the research group.

Processing of data was made using laboratory methods – common blood test – to define the degree of blood loss and

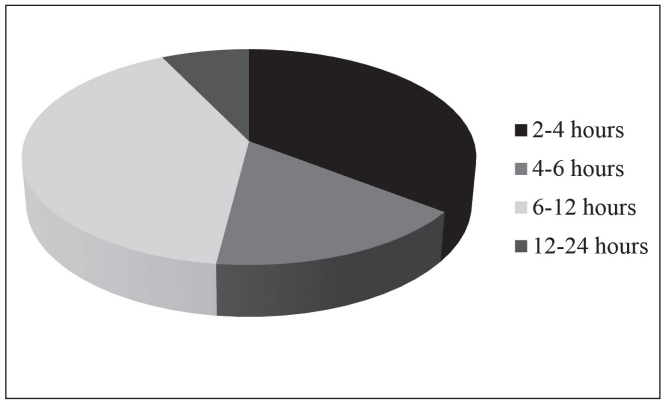

Fig.1. Distribution of patients with gastro-duodenal ulcerative hemorrhages depending on the extention of the pre-hospital period during the first day.

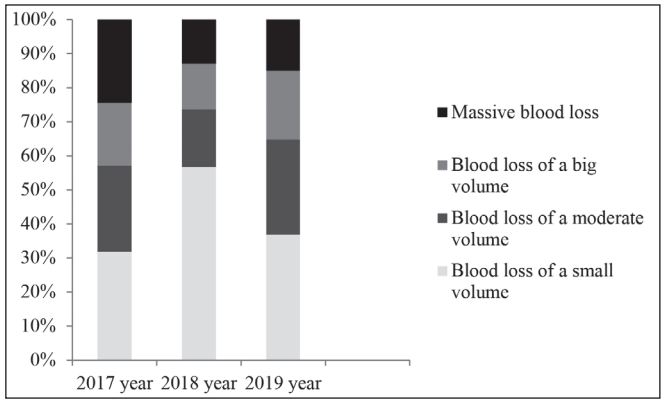

Fig. 2. Dynamics of distribution degrees of blood lost during the treatment of the background disease according to standard hypotension therapy in 2017–2019 years.

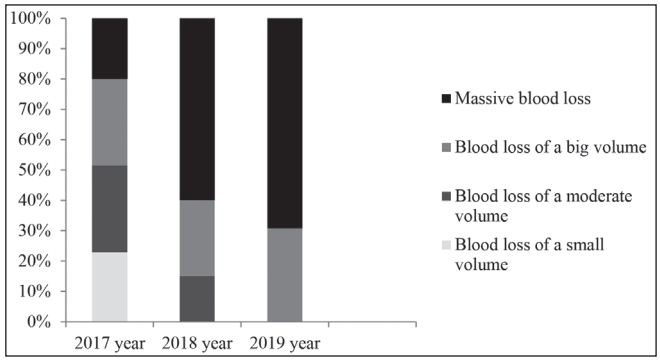

Fig. 3. Dynamics of distributing degrees of blood lost during the treatment of the background disease according to "double" scheme of therapy in 2017–2019 years.

main indexes of the immune system – IL-6, IL-10, TNF-α. Quantification of concentration of IL-6, IL-10, TNF-α in blood serum was carried out using enzyme-linked immunoassay by test systems [5].

General clinical method include esophagogastroduodenoscopy for determining the localization, size and condition of the local hemostasis.

Patient distribution analysis was carried out depending on the degree of blood loss according to the classification of American Association of Surgeons (1998) and it was revealed that severe degrees of blood loss (heavy and massive) were found in 234 patients (38.4%).

At analysis of the condition of the endoscopic hemostasis it was revealed that active hemorrhage F I – was observed in 38 patients (6.24%±0.98). Major part includes patients with signs of unstable local endoscopic hemostasis with a high risk of bleeding relapse – 486 patients (79.80%±1.63), ulcerative defect without signs of hemorrhage – 85 patients (13.96%±1.40).

All input data obtained during the research, with the purpose of optimizing mathematical processing were input in the database, which was built using spreadsheet Microsoft Excel. Statistical processing of the research results was made according to methods of variation statistics, implemented by the standard package of application programs Statistica for Windows 6.0. Descriptive statistics was used for the statistical analysis: M – mean value, m – error in determining the mean, comparison of mean values of variables carried out by the parametric method (Student t-test). Compliance of the type of distribution of characteristics with the law of normal distribution was checked using Shapiro-Wilk method. In other cases non-parametric method (Mann-Whitney U-test) was used. Difference of mean values of indices was considered reliable at $p<0.05$, $p<0.01$, $p<0.001$ [6].

RESULTS

Medical case histories of patients were analyzed. It was determined that from all 609 patients, who were hospitalized into the city center of hemorrhage 314 patients (51.6%) had ulcerative duodenal hemorrhages, 295 (48.4%) – gastric ulcer.

In 375 cases, pre-hospital period were 24 hours. It was revealed that within 2-4 hours 133 (35.47%±2.47) patients had been delivered to the in-patient department, within 4–6 hours – 62 (16.53%±1.92), within 6–12 hours – 153 (40.80%±2.54), within 12–24 hours – 27 (7.20%±1.33) patients .

Blood lost of a big amount and massive blood lost were noticed in 113 (18.56%±1.58) and 121 (19.87%±1.62) patients respectively. Blood lost of middle volume – 139 (22.82%±1.70) cases whereas due to certain reasons the small blood lost was revealed in 236 (38.57%±1.97) patients. Providing analysis the index of severity of blood lost, we determined that it directly depends on therapy, which the patients receive for treating pathologies of the cardio-vascular system.

Having an analysis the degree of blood lost depending on the therapy aimed at a cardio-vascular pathology, in particular in case of receiving standard hypotension therapy (n=541), it is possible to conclude that a small blood loss prevailed 228 (42.14%±2.12) along with moderate blood lost – 126 (23.29%±1.82) cases. Blood lost of a big volume and massive blood lost were 94 (17.38%±1.63) and 93 (17.19%±1.62) patients respectively. In 2018 the blood lost of a small volume was 105 (56.8%) cases, in comparison with 2019 – 34 cases more and it is 20% ($p<0.05$).

During the "double" therapy (n=68), blood loss of a big volume and massive blood lost were noticed more often

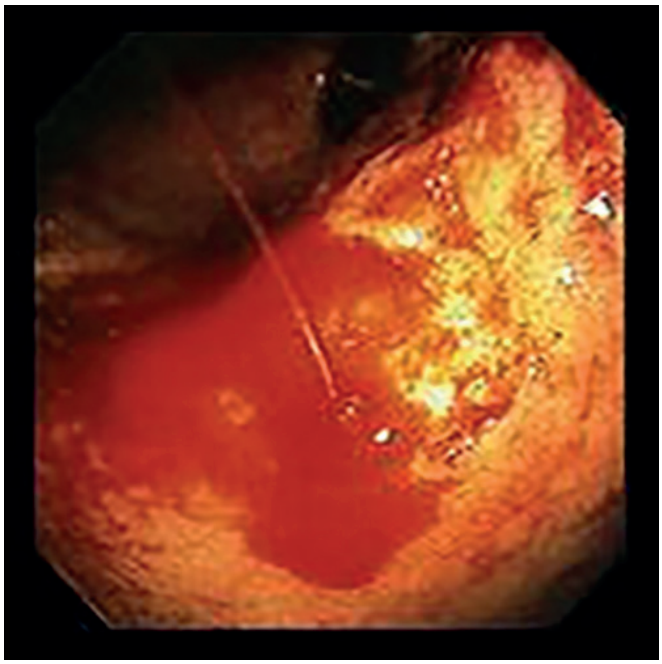

Fig. 4. Active hemorrhage. F IA.

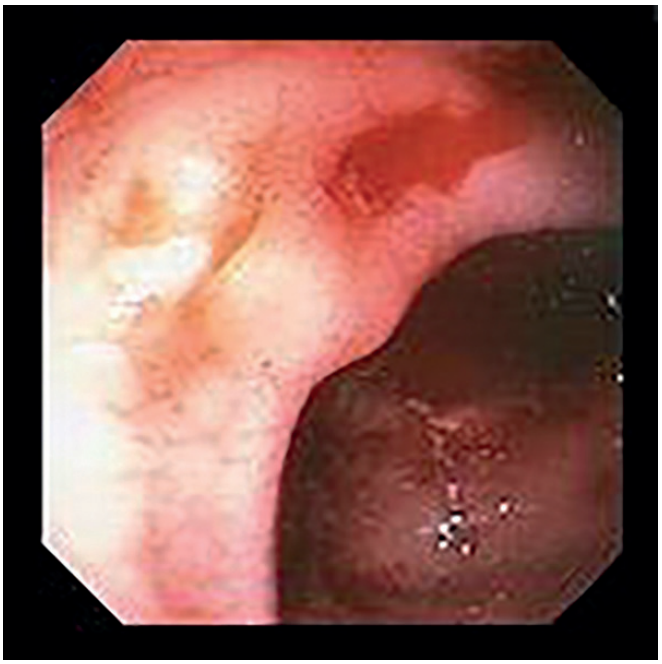

Fig. 5. Blood leakage. F IB.

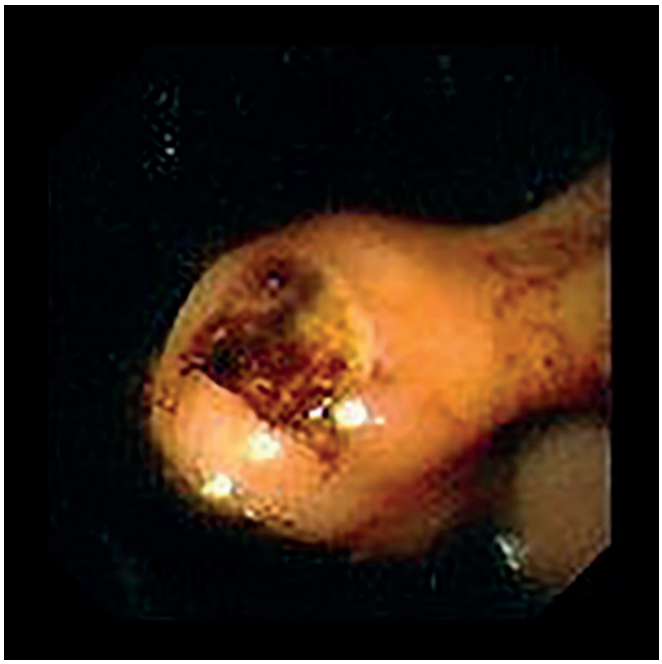

Fig. 6. Visible thrombosed vessel. F IIA

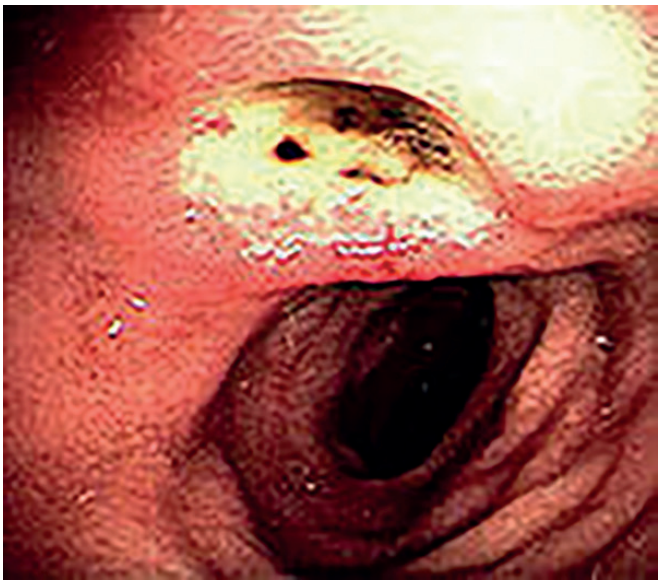

Fig. 7. Signs of recent hemorrhage. F III

– 19 (27.94%±5.44) and 28 (41.18%±5.97) patients respectively. Blood lost of a small volume and blood lost of a moderate volume were 8 (11.76%±3.91) and 13 (19.12%±4.77) cases respectively. Massive blood lost was 69.2% in 2019, which is 9.2% more than that of 2018; blood lost of a small volume was not revealed during this period (p>0.05).

Local endoscopic hemostasis was considered according to Forrest classification. Active increase (F I) was revealed in 38 patients (6.24%±0.98) among them FIA – 18 (2.96%±0.69), FIB – 20 (3.28%±0.72).

A high risk of hemorrhage relapse was determined in 486 patients (79.80%±1.63), at that F IIA – in 161 patients (26.44%±1.79), F IIB – in 191 patients (31.36%±1.88), F IIC – in 134 patients (22.00%±1.68). Signs of recent hemorrhage were absent in 85 patients (13.96%±1.40)

DISCUSSION

The analysis has shown that the indicators of the local endoscopic hemostasis depends on therapy aimed at treatment of the cardiovascular pathology; it was revealed that within 2017-2019, in case of receiving standard hypotension therapy the endoscopic picture of F III prevailed – 89 (16.4%) cases, and F I was revealed in 27 (5%) patients. In 2019 F III revealed in 19.7% patients, which is 4% more than that of 2018 and 6.2% more than in 2017. Active hemorrhage

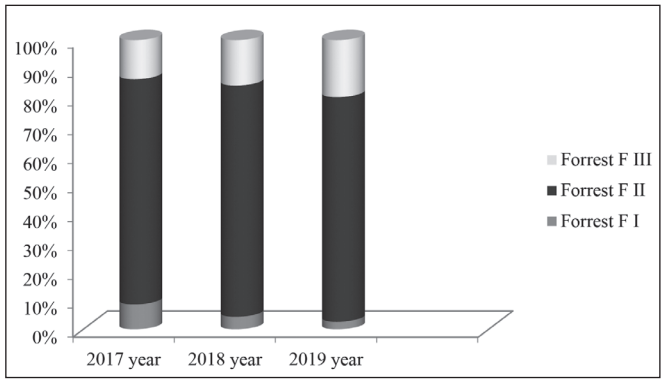

Fig.8. Dynamics of distributing local endoscopic hemostasis at treatment of the background pathology with the help of the hypotension therapy.

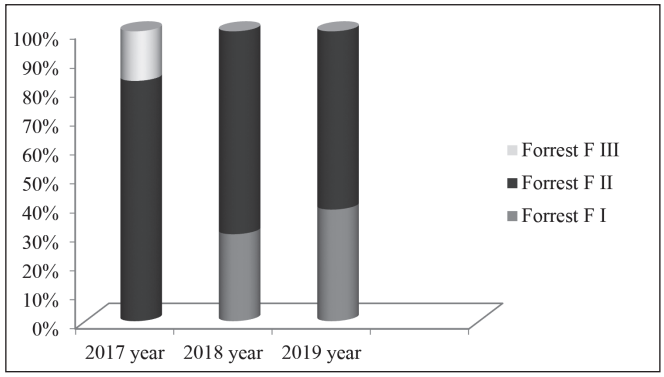

Fig.9. Dynamics of distributing local endoscopic hemostasis at treatment of the background pathology with the help of the "double" therapy.

F I, in its turn tends to decrease in 2019 by 6%, than the same indicator in 2017 (p<0.05).

Having analyzed the indices of the local endoscopic hemostasis in patients who receive "double" therapy for treating a cardiovascular pathology, we can conclude that indicator of active hemorrhage significant increased in 2018 in comparison with 2017 – by 30% with consequent gradual increase in 2019 – by 8.5%

Indicator F III remarkable decreased in. Moreover, in 2019 there were no patients with F III (p<0.05).

At all stages of forming specific immune response of the body, the dominant role belongs to cytokines. Increase in the level of cytokines is an essential component of adequate reaction of the body to inflammation [7, 8, 9]. At the same time, over expression of mediators causes changes of physiological processes. The research of cytokines in patients with gastroduodenal hemorrhage has been carried out Tab.I.

Analysis of the obtained research results has show that patients of the II group have an increased level of IL-6 in blood serum – in 47.9 % (p>0.05), and TNF-α (2.4 times) – in 43.8 % patients (p<0.05). Level of IL-6 in blood serum of the II group of patients was significantly increased 1.8 times, (p<0.05) in comparison with values in the I group of patients. It illustrates the activity of the inflammatory process in patients of the II group. The level of anti-inflammatory IL-10, which slows down the proliferative response of T-cells, was within the norm. The correlation link between levels of IL-6 in blood serum and the level of and polymorphonuclear leukocytes (r=+0.4; p<0.01), the intensity of hemorrhage (r=+0.29; p<0.05), with the level of TNF-α in blood serum (r=+0.64; p<0.01) and the level of IL-10 in blood serum (r=+0.45; p<0.01) was determined.

In the II group of patients there was an increase in the level of IL-6 (p>0.05) in blood serum in 28.6 % patients, TNF-α – (1.6 times) in 28.6 % (p<0.05). The number of IL-10 in patients of the II group was within the norm.

The correlation link was determined between the level of IL-10 and inflammation activity (r=-0.76; p<0.01), level of IL-6 (r=+0.64; p<0.05), level of TNF-α (r=+0.76; p<0.01). The level of anti-inflammatory IL-6 correlated with: the level of TNF-α (r=+0.86; p<0.01). A high level of pro-inflammatory cytokines IL-6, TNF-α and a low activity of the anti-inflammatory mediator IL-10 define the process activity, their long-term circulation in patients with ulcerative hemorrhages from the upper areas of the gastro-intestinal tract are associated with unfavorable prognosis. In case of imbalance between pro- and anti-inflammatory mediators in favor of the first, the risk for relapsing hemorrhage in the second group increases. Changes in the number of anti-inflammatory cytokines IL-6, TNF-α in peripheral blood could be the reason and one of the realization mechanism of hemorrhage relapse.

During the period 2017-2019 years 10 elderly patients (1.6%) were operated. In 5 cases conditionally-radical surgical interventions were performed. They include excision of the ulcerative defect with pyloroplasty by Heyneke-Mikulicz (30%), by Finney (20%). In this case ulcerative defects were localized in duodenum. Palliative surgery included suturing of ulcerative defect with truncal vagotomy (TV) – 3 patients. Partial gastric resection performed in 2 cases. Ulcerative defects localized in duodenum and

Table I. Level of cytokines in patients with gastroduodenal hemorrhages, Me (Q1; Q2).

Indicators, units of measurement	I group (n=541)	II group (n=68)	Control group (n=20)	p_1	p_2	p_3
TNF-α, mμg/ml	3.5 (0.27; 7.4)	5.4 (1.1; 15)	2.3 (0.3; 3.9)	p<0.05		
IL-6, mμg/ml	3.7 (0.8; 9.8)	5.38 (3.01; 21.9)	5.60 (1.2; 7.8)			p<0.05
IL-10, mμg/ml	17.1 (11.4; 25.1)	16.9 (12.8; 24.2)	18.7 (0.4; 21.4)			

Remark:

1. p_1 – significance of differences of the I group of patients in comparison with the control group;
2. p_2 – significance of differences of the II group of patients in comparison with the control group;
3. p_3 – significance of differences between I and II group of patients.

stomach in ratio 1:2 (p>0.05). Differences were insignificantly reliable due to small number of patients.

Post-operative complications included the relapse of hemorrhages, suture failures of the stitched area of ulcer. Post-operative mortality was 30%, which testifies in favor of the initially severe condition of patients, complicated by cardio-vascular pathology. Most patients operated on the peak of hemorrhage and could be considered as a "surgery of despair".

CONCLUSIONS

1. An essential aspect for choosing the medical tactics include taking into consideration the cardio-vascular pathology.
2. The number of second group patients ("double therapy") with big and massive blood loss is 2.7 times higher than similar indices in of the first group (standard therapy) (p<0.05).
3. According to analysis of the condition of the local endoscopic hemostasis, the indicator of the unstable hemostasis with the high risk for hemorrhage relapse, with stable hemostasis F III in I group is 1.8 times higher than the similar indicator in the II group (p<0.05). These data have been the ground of treatment elaboration. As for the index of active hemorrhage F I, the patients who receive standard hypotension therapy have 3.3 times less active hemorrhage than the patients who receive "double therapy" (p<0.05).
4. In the group of patients who had hemorrhage relapse and received "double therapy", indices of body immune reactivity are compared to the group of patients who have got standard hypotension therapy and have smaller hemorrhage relapse rate which coming along more positive tendency of immune status changes.
5. In cases of acute cardiovascular pathology the palliative surgery was performed in 100%. At the same time the part of the conditionally-radical methods, including elements of radicalism and organ-preserving type is increased.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

ANATOMICAL VARIABILITY OF CUTANEOUS NERVES OF ANTERIOR FEMORAL REGION IN HUMAN FETUSES

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ABSTRACT

The aim: Is to find out the features of innervation of the skin of the anterior femoral region and the fascia lata during the fetal period of human development.

Materials and methods: The study was carried out on 64 preparations of the lower extremities of human fetuses of 4-10 months using macromicroscopic preparation and morphometry. Macropreparations of the skin nerves of the lower extremities of different age fetuses with anatomical variants were subject to photo documentation.

Results: The features of cutaneous nerve fetal topography of the anterior femoral region and the broad fascia of the femur were revealed, their connections were established, and their layering was determined. It was found that in human fetuses, not only the lateral cutaneous femoral nerve but in most cases the branches of other nerves of the lumbar plexus, except for the obturator nerve, are directed to the skin of the anterior-lateral femur surface. The innervation of the medial femur surface is provided by the following nerve complex: obturator, femoral, saphenous and genitofemoral nerves.

Conclusions: Taking into account the fact that the terminal branches of adjacent cutaneous nerves of the femoral region intersect and overlap, innervation bypasses are formed, due to which, in case of possible damage to one of the nerves, its insufficiency is compensated to a certain extent.

Anastomoses were found between the cutaneous nerves, in the form of loops of various shapes and sizes, namely: between the cutaneous-fascia branches of the femoral and ilioinguinal nerves and the femoral and obturator nerves.

KEY WORDS: skin, innervation, femoral region, fetus, human

Wiad Lek. 2021;74(2):207-212

INTRODUCTION

Connective tissue formations, which are considered as a flexible soft skeleton of the human body, are important In the vital activity of the body. The soft skeleton contains formations with loose and dense connective tissue. Loose connective tissue is under the skin. More diverse formations of the soft skeleton are built of dense connective tissue – fascia, tendons, ligaments, capsule joints, fibrous sheaths of the neurovascular bundles and the like. The priority of fascia, in particular the fascia lata, is well-known in plastic surgery [1, 2, 3]. Elasticity, relative flexibility and fitness are inherent for fascia lata, which makes it possible to model it. However, data on the features of innervation of the fascia lata during the fetal period of human ontogenesis remain insufficiently studied in the research literature, and they remain fragmentary and contradictory [4].

Studies of individual authors were devoted to the variant anatomy of the lateral cutaneous nerve of the femur [5-10]. In particular, based on the lateral femoral cutaneous nerve topography study in 20 corpses by A. Hanna [9], it was found that coming out from the lateral edge of the psoas major muscle, the nerve crosses the iliac muscle and passes under the inguinal ligament at femur medial (6.5 cm) or lateral (6.0 cm) of the anterior superior iliac spine. It is indicated that the lateral cutaneous nerve of the femur passes in the fascia canal. Also, in isolated cases, the lateral femoral cutaneous nerve runs along the anterior superior iliac spine, can be doubled, or start from the femoral nerve.

Usually, the lateral femoral cutaneous nerve runs laterally relative to the sartorius [6]. Some authors point to the bifurcation of the lateral femoral cutaneous nerve in anterior and posterior branches that innervate the skin of the anterolateral, lateral and posterior-lateral surfaces of the femur, including the greater trochanter region [5, 11, 12].

Literature data suggest reports on the ultrasound anatomy of the cutaneous nerves of the extremities [12-14]. Thus, K.V. Chang et al. [13] describe the anterior femoral cutaneous nerve as a branch of the femoral nerve. The anterior femoral cutaneous nerve is divided into intermediate and anterior branches. The intermediate branch of the anterior femoral cutaneous nerve passes through the femur fascia lata, crosses the sartorius and innervates the skin of the anterior femoral and knee sections, and its anterior branch descends obliquely along the sartorius, penetrates the fascia in the distal third of the femur and provides innervation of the skin in the medial part of the anterior femoral region. Both branches of the anterior femoral cutaneous nerve can anastomose with lateral femoral cutaneous nerve, obturator nerve and patella branch of the saphenous nerve, forming the plexus [15].

The saphenous nerve is a direct extension of the femoral nerve and its terminal branch. It accompanies the femoral

artery in the anterior femoral sulcus and in the adductor canal. The saphenous nerve exits the adductor canal along with the descending knee artery and penetrates the femoral fascia lata [12, 13].

Thus, the literature sources do not sufficiently cover the features of innervation of the skin of the anterior femoral region and the fascia lata during various periods of human ontogenesis, in particular in different age fetuses.

THE AIM

The aim is to find out the features of innervation of the skin of the anterior femoral region and the fascia lata during the fetal period of human development.

MATERIALS AND METHODS

The study was carried out on 64 preparations of the lower extremities of human fetuses of 4-10 months using macromicroscopic preparation and morphometry. Macromicroscopic preparation of the branches of the lumbar and sacral plexus was carried out using a binocular magnifier. First, the lumbar and sacral plexus nerves in the pelvic area were prepared, and then their cutaneous nerves or (and) cutaneous branches that go to the skin of the anterior and inner surfaces of the femoral region were prepared. It is necessary to note that in the pelvic region, the lumbar plexus nerves were prepared in two steps: first, the branches of the lumbar plexus were dissected with the preservation of the large lumbar muscle, and then after the latter was removed. Inguinal ligament was preserved in all cases. In order not to damage the cutaneous nerves of the femoral region of branches that intersect with each other, we followed the sequence of preparation of the nerves of the lumbar plexus. First, the iliohypogastric and ilio-inguinal nerves were prepared, then the branches of the ilio-inguinal and genitofemoral nerves were prepared, and after that, the lateral femoral cutaneous nerve, femoral and obturator nerves were prepared. The selected sequence of the cutaneous nerves of the anterior femoral region preparation made it possible to reveal a shift in the innervation zones, to establish overlap zones and other forms of variability by the topography of the nerve trunks and their branching in the skin of the femoral area. During the step-by-step preparation of branches of the lumbar plexus, variants of the structure and topography of cutaneous nerves or (and) cutaneous branches were found. Macropreparations of the skin nerves of the lower extremities of different age fetuses with anatomical variants were subject to photo documentation.

RESULTS

As a result of the study, the features of the fetal topography of individual cutaneous nerves of the anterior femoral region were identified and their connections were set, as well as the layered arrangement of their location. In our opinion, the layered arrangement of nerve trunks that provide innervation of the skin of the anterior femoral region is associated with the level of formation of the lumbar plexus nerves from the anterior branches of the spinal nerves, namely: the higher the level of the main nerve trunk origin, the more superficially its branches are placed in the subcutaneous fat and vice versa, the lower the nerve originates, the more deeply its branches are directed.

Taking into account the above, three cutaneous nerve sets can be distinguished in the anterior femoral region. One set is located on the anterior femoral surface, the anterior cutaneous branches of the femoral nerve, lateral femoral cutaneous nerve, the anterior cutaneous branch of the Iliohypogastric nerve, ilio-inguinal and genitofemoral nerves are involved in its formation (Fig. 1, 2). Innervation of the skin of the upper third of the lateral femoral surface is provided by a complex of cutaneous nerves, which is mainly formed by the lateral cutaneous branch of the Iliohypogastric nerve and the lateral femoral cutaneous nerve. Also, the anterior cutaneous branches of the femoral nerve and the genitofemoral nerve participate in the innervation of the skin of the lateral femoral surface. It should be also noted that the lateral femoral cutaneous nerve below the inguinal ligament, as a rule, branches into 2-5 branches, which provide innervation of the lateral femoral surface skin to the knee (Fig. 3). At the same time, the cutaneous nerves or branches that innervate the skin of the anterolateral femoral surface, when exiting the femur, are mainly located behind the lateral and medial thirds of the inguinal ligament.

The following set of nerves is involved in the innervation of the medial femoral surface skin: 1-3 cutaneous branches of the obturator nerve, anterior cutaneous branches of the femoral nerve, saphenous and genitofemoral nerves (Fig. 4-6). Usually, 1-3 femoral branches depart from the latter to the skin of the upper medial surface of the femur under the inguinal ligament. The cutaneous branches of the obturator nerve mainly innervate the skin of the lower part of the femur inner surface. In the innervation of the femur skin of the thigh on the border of its front and rear surfaces can also participate lateral femoral cutaneous nerve, the femoral branch of the genitofemoral nerve, anterior cutaneous branches of the femoral nerve and cutaneous branch of the obturator nerve.

It was found that the lateral femoral cutaneous nerve formed connections with the anterior cutaneous branches of the femoral nerve at different levels of the femoral region. Anterior cutaneous branches of the femoral nerve, in addition to the above relations, also are connected with the cutaneous branches of the obturator nerve within the middle and lower thirds of the femoral internal surface, and the saphenous nerve in the femoral triangle region and anterior medial surface of the lower third of the femur in the region of the patella. The saphenous nerve is connected with the cutaneous branch of the obturator nerve at different levels: up to the adductor canal, in the canal itself, and in the lower third of the femur.

The superficial fascia on the anterior medial surface of the femur forms fascia cases for the cutaneous nerves and

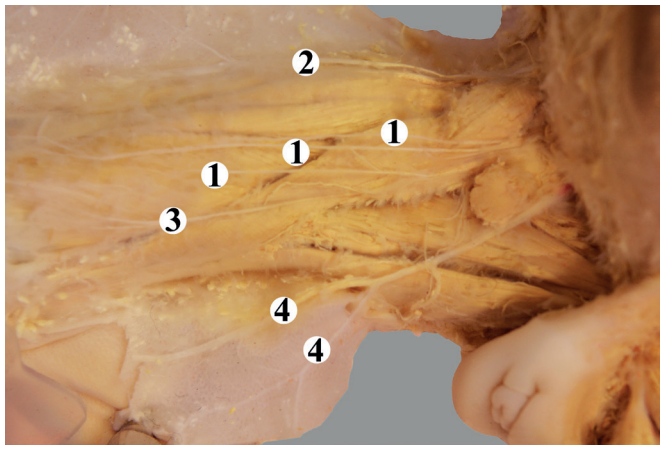

Fig. 1. Vessels and nerves of the right anterior femoral region of the female fetus – 285.0 mm of PCL. Macrospecimen. Mag. 2.4x:
1 – anterior cutaneous branches of the femoral nerve; 2 – lateral femoral cutaneous nerve; 3 – subcutaneous nerve; 4 – cutaneous branches of the femoral artery.

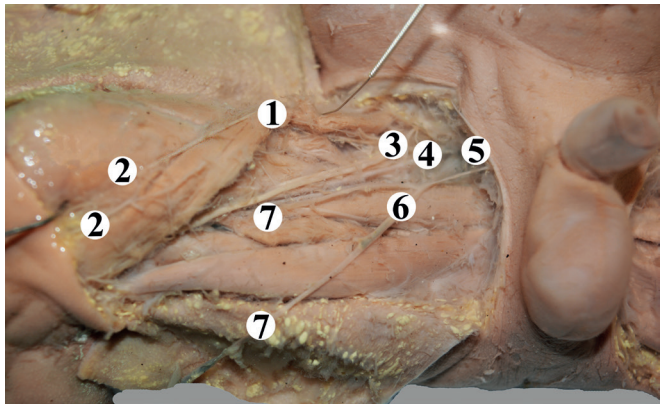

Fig. 3. Vessels and nerves of the right anterior femoral region of the male fetus – 265.0 mm of PCL. Macrospecimen. Mag. 2.5x:
1 – lateral femoral cutaneous nerve; 2 – branches of the lateral femoral cutaneous nerve; 3 – femoral nerve; 4 – femoral artery; 5 – femoral vein; 6 – great saphenous vein; 7 – cutaneous tributaries of the great saphenous vein.

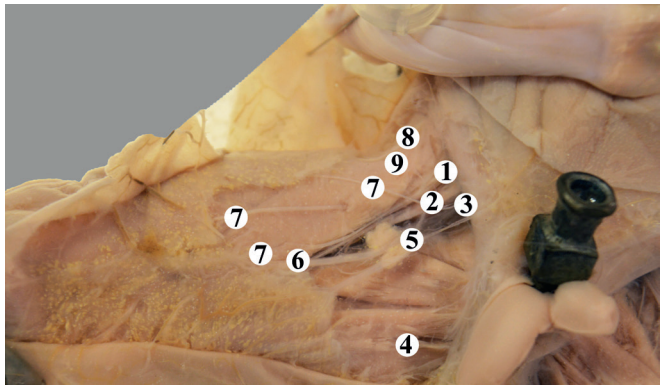

Fig. 5. Vessels and nerves of the right anterior femoral region of the male fetus – 240.0 mm of PCL. Macrospecimen. Mag. 2.1x:
1 – femoral nerve; 2 – femoral artery; 3 –femoral vein; 4 – obturator nerve; 5 – lymph node; 6 – great saphenous vein; 7 – branches of the femoral nerve; 8 – lateral femoral cutaneous nerve; 9 – connecting branch between the lateral femoral cutaneous nerve and the femoral nerve.

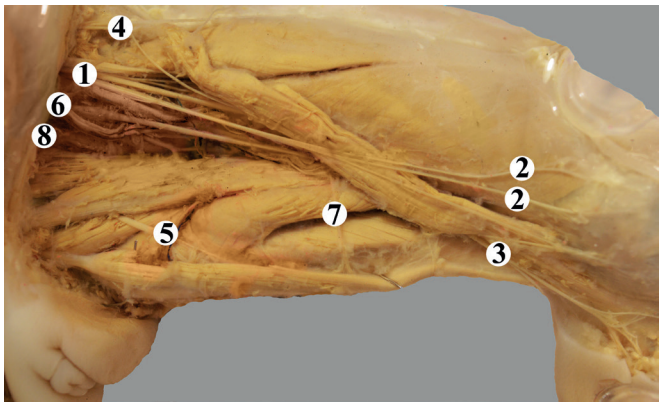

Fig. 2. Vessels and nerves of the left anterior femoral region of the female fetus – 285.0 mm of PCL. Macrospecimen. Mag. 2.1x:
1 – femoral nerve; 2 – anterior cutaneous branches of the femoral nerve; 3 – subcutaneous nerve; 4 – lateral femoral cutaneous nerve; 5 – obturator nerve; 6 – femoral artery; 7 – muscular branch of the femoral artery; 8 – femoral vein.

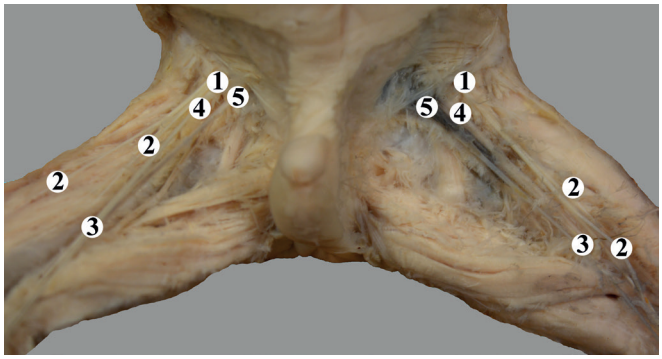

Fig. 4. Vessels and nerves of the anterior femoral regions of the male fetus 272.0 mm PCL. Macrospecimen. Mag. 1.8 x:
1 – femoral nerve; 2 – anterior cutaneous branches of the femoral nerve; 3 – subcutaneous nerve; 4 – femoral artery; 5 – femoral vein.

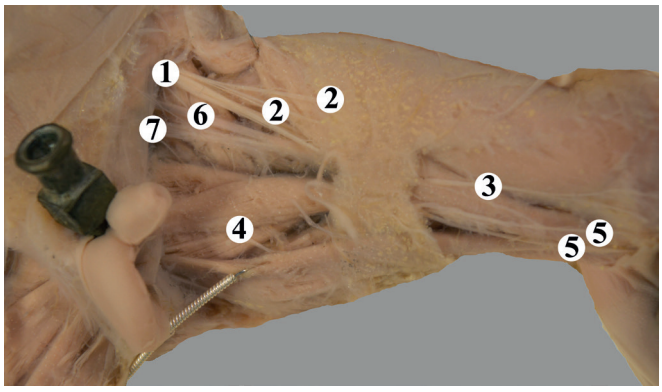

Fig. 6. Vessels and nerves of the left anterior femoral region of the male fetus – 240.0 mm of PCL. Macrospecimen. Mag. 2.2x:
1 – femoral nerve; 2 – anterior cutaneous branches of the femoral nerve; 3 – subcutaneous nerve; 4 – obturator nerve; 5 – obturator nerve branches; 6 – femoral artery; 7 – femoral vein.

the great saphenous vein. Within the upper third of the femoral triangle, the superficial fascia loosely fuses with the inguinal ligament and the surface plate of the femur fascia lata. Fascia sheath of the great saphenous vein in

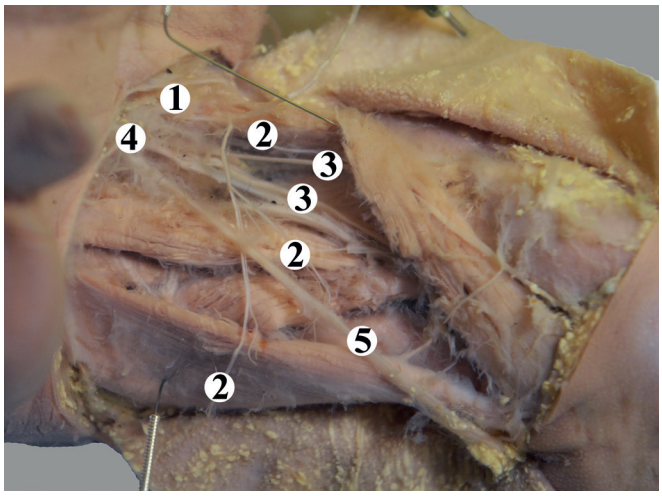

Fig. 7. Vessels and nerves of the left anterior femoral region of the male fetus – 265.0 mm of PCL. Macrospecimen. Mag. 2.3 x:
1 – ilio-inguinal nerve; 2 – cutaneous branches of the ilio-inguinal nerve; 3 – branches of the femoral nerve; 4 – femoral vein; 5 – great saphenous vein.

the upper third of the femur formed by splitting of the superficial fascia or the fascia lata plate, and the medial and lower thirds of the femur that vein is located in the splitting of the plates of the fascia lata. Superficial inguinal lymph nodes are located on both sides of the great saphenous vein at its entry to the femoral vein. The fascia cases of the latter are loosely connected to the fascial sheath of the great saphenous vein. It should be noted that over the fetal period of ontogenesis, the fascia lata along its length is of unequal structure. In the proximal part of the anterior femoral region, the fascia lata is poorly developed in different age fetuses. In the area of the femoral triangle, when moving from the sartorius to the adductor muscles, the fascia lata is divided into superficial and deep plates and forms fascial cases for the superficially located femoral muscles: muscle tensioner of the fascia lata, sartorius, thin muscle and rectus femoris. The surface plate of the fascia lata, in turn, is divided into several thin, loose plates that can not be distinguished, because fat inclusions are found in the surface fascia and the surface plate of the fascia lata. There is an oval fossa in the surface plate of the fascia lata. In the studied fetuses, the fascia lata plates are transparent and loose, with the exception of the outer surface of the femur, where the fascia lata is slightly compacted and forms an iliotibial strand. In 8-10 month fetuses, the broad fascia throughout the posterior femoral region, primarily within the popliteal fossa, is strengthened by bundles of fibers, mainly in a transverse direction.

The branches of the lumbar and sacral plexus are the source of the fascia lata innervation. In particular, 3-6 cutaneous fascial branches extend from the femoral nerve below the inguinal ligament, which penetrate the fascia lata and branch out within the anteromedial femoral surface. 2-3 branches are directed to the fascia and skin of the upper third of the medial femur surface from the ilio-inguinal nerve below the inguinal ligament (Fig. 7).

As a rule, 1-2 branches and sometimes three branches of the obturator nerve are involved in the innervation of the skin of the medial femoral surface. In some cases, 1-2 skin-fascial branches of the obturator nerve reach the level of the lower third of the medial femur surface.

The lateral femoral cutaneous nerve below the anterior superior iliac spine gives off 2-3 branches involved in the innervation of the fascia lata and skin of the lateral and partially anterior femoral surfaces. 2-4 branches of the posterior cutaneous femoral nerve are involved in the innervation of the fascia lata and skin of the posterior femoral region.

The terminal branches of the femoral nerve overlap the cutaneous-fascial branch of the obturator nerve, the branches of the lateral femoral cutaneous nerve, as well as the branches of the iliac-inguinal nerve on the lateral surface – the branches of the lateral femoral cutaneous nerve, on the medial femoral branch surface. The latter also forms "overlapping zones" with branches of the posterior cutaneous nerve on the posterior femoral surface, and in some cases, in the area of the lateral femoral surface. Together with the venous and arterial vessels that accompany the branching of the above-mentioned nerves, a neurovascular network is formed in the thickness of the connective tissue of the fascia lata.

Anastomoses in the form of loops of various shapes and sizes were found in the anterior-medial femoral surface region of the studied fetuses, namely: between the cutaneous-fascia branches of the femoral and ilioinguinal nerves (within the upper third) and the femoral and obturator nerves (within the middle third).

DISCUSSION

It should be noted that the textbooks and manuals on anatomy indicate that in the skin of the lateral and anterior-lateral femoral surfaces the lateral femoral cutaneous nerve divides, the skin of the upper-medial femoral surface under the inguinal ligament is innervated by the femoral branch of the genitofemoral nerve, the skin of the lower portion of the medial femoral surface – by the cutaneous branch of the obturator nerve and anterior cutaneous branches of the femoral nerve innervate the anterior femoral surface skin [9, 16]. However, as a result of the study, anatomical variability in the innervation of the skin of the anterior femoral region was found. It was also found that in human fetuses, not only the lateral cutaneous femoral nerve but in most cases the branches of other nerves of the lumbar plexus, except for the obturator nerve, are directed to the skin of the anterior-lateral femur surface. The innervation of the skin of the anterior-lateral femur surface is provided by a cutaneous nerve complex: the femoral nerve, the lateral femoral cutaneous nerve, iliohypogastric, ilioinguinal and genitofemoral nerves. The innervation of the medial femur surface is provided by the following nerve complex: obturator, femoral, saphenous and genitofemoral nerves. Based on a thorough anatomical study of the branching zones of the cutaneous nerves in the anterior femoral region and the fascia lata,

both the displacement of the innervation zones and the overlap zones were set. It was found that as a result of branching of the ilioinguinal branches of the femoral, obturator, iliac-inguinal, lateral and posterior cutaneous nerves of the thigh, "overlapping zones" of one nerve by the other are formed on the entire surface of the fascia lata. Therefore, the terminal branches of these nerves involved in the innervation of the fascia lata do not have a well-defined topography.

The obtained data on the variant anatomy of the cutaneous nerves and the cutaneous-fascial branches of the nerves of the anterior femoral region should be taken into account by surgeons during surgical interventions. As K.A. Tomaszewski et al. [7] stress that knowing the possible options for the topography of the lateral cutaneous nerve will help reduce the risk of nerve damage during surgery within the inguinal and femoral region, in particular during hip replacement and inguinal herniotomies.

The results of the study are also of great practical importance for neurosurgery, neurology and reflexology.

CONCLUSIONS

In human fetuses, anatomical variability of the cutaneous nerves and cutaneous branches of the anterior femoral nerve region was established, and three sets of cutaneous nerves were identified.

Anastomoses were found between the cutaneous nerves, in the form of loops of various shapes and sizes, namely: between the cutaneous-fascia branches of the femoral and ilioinguinal nerves (within the upper third) and the femoral and obturator nerves (within the middle third).

Taking into account the fact that the terminal branches of adjacent cutaneous nerves of the femoral region intersect and overlap, innervation bypasses are formed, due to which, in case of possible damage to one of the nerves, its insufficiency is compensated to a certain extent.

In human fetuses, innervation of the fascia lata in the anterio-medial femoral surface is provided by branches of the femoral, obturator, and ilio-inguinal nerves, and in the posterior-lateral surface of the femur – branches of the posterior and lateral cutaneous nerves of the femur.

Connections and complexes of femoral cutaneous nerves, as well as overlapping and displacement zones are compensatory mechanisms in the peripheral nervous system and are observed not only between ontogenically related nerves, but also nerves of various segmental affiliation.

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The study is a part of the planned complex topic at the Department of Histology, Cytology and Embryology of the HSEI of Ukraine Bukovynian State Medical University named "Regularities of Morphogenesis, Structural and Functional Features of Tissues and Organs in Human Ontogenesis", state registration No. 0116U002938.

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The Authors declare no conflict of interest.

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

ORIGINAL ARTICLE

CD68+ M1 MACROPHAGES IS ASSOCIATED WITH PLACENTAL INSUFFICIENCY UNDER FETAL GROWTH RESTRICTION

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ABSTRACT

The aim: To elucidate the possible involvement of M1 and M2 macrophages in the placentas of women, whose pregnancies were complicated by fetal growth restriction (FGR) and resulted in term births after 37 weeks of gestation and preterm births up to 37 weeks of gestation.

Materials and methods: CD68+ and CD163+ macrophages were studied by immunohistochemical method, placental morphology in the placentas of 16 women whose pregnancies were complicated by FGR and resulted in term births at a gestational age after 37 weeks (1-st group, n = 7) or resulted in preterm births at a gestational age up to 37 weeks (2-nd group, n = 9). The control group consisted of 10 placentas of women with physiological pregnancies and births.

Results: Women 2-nd group showed significantly low weight of the placenta, a short gestation period at the time of delivery, and a prolonged labor period than women of the control group ($p < 0.001$; $p < 0.001$; $p < 0.05$, respectively). The level of CD68+ and CD163+ macrophages in the placentas of women 2-nd group was significantly higher than in woman 1-st group ($p < 0.001$, $p < 0.001$, respectively). A significant correlation was found between the expression level of CD68+ monocytes in the intervillous space and the weight of a newborn ($r = -0.765$; $p = 0.016$) in women 2-nd group.

Conclusions: These studies suggest that in the placentas of women whose pregnancies were complicated by FGR and resulted in preterm births, the increased activation of CD68+ macrophages of the pro-inflammatory pool may be associated with disorders of the vascular and stromal component of the villous chorion with the development of involutive and dystrophic changes. In general, this fact probably determines the progress of chronic placental insufficiency and aggravates the development of fetal growth restriction.

KEY WORDS: fetal growth restriction, preterm and in term birth, preterm and in term delivery, placental insufficiency, morphology of placenta, M1 and M2 macrophages

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INTRODUCTION

Fetal growth restriction (FGR) is an urgent problem of modern medicine that occurs in 3-8% of all pregnancies [1]. FGR is accompanied by an increasing risk of preterm birth, perinatal diseases and mortality, as well as an unfavorable course of adaptation processes in newborns [2]. In FGR, functional insufficiency of the placenta is observed, which is often registered during early termination of pregnancy in the form of preterm birth. Placental insufficiency is combined with structural and functional disorders, among which the inflammatory and immune processes are of particular importance [3]. Inflammation and activation of immune cells of the monocyte-macrophage system constitute an integral component of the major gestational events in physiological and pathological pregnancy, in particular under FGR.

Macrophages (Mφs) play a key role in the immune defense and maintenance of homeostasis [4, 5]. Monocyte-macrophage system is distinguished by a high degree of heterogenenicity and plasticity, depending on the microenvironmental factors. There are 2 types of Mφs – M1 ("the classically activated type", pro-inflammatory) and M2 ("the alternatively activated type", anti-inflammatory). Mφs in the placenta is associated with the involvement of these cells in the physiological and pathogenetic processes of pregnancy and childbirth at all stages. During physiological pregnancy, macrophages are balanced in the immunoregulatory phenotype and regulate the innate and adaptive immune response. It is obvious that Mφs (decidual and Hofbauer cells) exhibit a predominantly anti-inflammatory M2 phenotype in the placenta. These Mφs are involved in the tissue remodeling and homeostasis, in the maintaining the maternal and fetal tolerance, trophoblast invasion, spiral artery remodeling, as well as tissue regeneration and angiogenesis [6,7].

The data on macrophages in FGR are very limited. It was revealed that CD68+ M1-macrophages have focal localization in the stroma of villi during pregnancy complicated by FGR. The number of Mφs is significantly higher in women whose pregnancies resulted in term births, in contrast to women whose pregnancies resulted in preterm births [8]. It was shown that the pro-inflammatory M1 type of Mφs is involved in the mechanisms of preterm birth [9]. At the same time, the role and phenotypic characteristics of M1 and M2 Mφs in the pathogenetic mechanisms of pregnancy, complicated by FGR and resulting in term or preterm birth, remain completely unexplored. It is obvious that the quality and adequacy of signal transduction by macrophages can determine the course of pregnancy, its outcome and timely prognosis. Therefore, macrophages require a particular attention when considering the pathogenetic features of FGR and represent an attractive therapeutic target for the prevention of arising disorders.

THE AIM

The aim of the current study was to elucidate the possible involvement of M1 and M2 macrophages in the placentas of women, whose pregnancies were complicated by fetal growth restriction (FGR) and resulted in term births after 37 weeks of gestation and preterm births up to 37 weeks of gestation.

MATERIALS AND METHODS

26 placentas of women whose pregnancy resulted in live birth at a gestational age from 24 to 41 weeks at the City Clinical Maternity Hospital of Poltava City Council in 2015–2017 comprised the material of the present study. Placental bed biopsies were collected from women, whose pregnancies were complicated by FGR and whose pregnancies resulted in term births after 37 weeks of gestation (1-st group, n=7), from women, whose pregnancies were complicated by FGR and whose pregnancies resulted in preterm births within 37 weeks of gestation (2-nd group, n=9) and from women with physiological pregnancies and births (control group; n=10). The study was approved by the Commission on Bioethics of Ukrainian Medical Stomatological Academy. Written consent was obtained from all study participants.

FGR was defined as birth weight less than 10th centile with an increased ratio of systolic / diastolic pressure in the umbilical artery by Doppler ultrasonography or resistance index> 95th centile for gestation. All patients with FGR

Table 1. Characteristics of women with physiological pregnancies (n = 10), women whose pregnancies were complicated by FGR and resulted in term births (n = 7), and women whose pregnancies were complicated by FGR and resulted in preterm births (n = 9)

Parameter	Clinical Characteristics of Pregnancies for Placentas Studied based Mean ± SD, median [IQR] or n (%)		
	Women with physiological pregnancies n=10	Women whose pregnancies were complicated by FGR and resulted in term births n=7	Women whose pregnancies were complicated by FGR and resulted in preterm births n=9
Gravidity	1 [1-3]	1 [1-2]	3 [1-4]
Parity	1 [1-2]	1 [1-1]	1 [1-2]
Gestational age (weeks)	38.9±0.99	38.86±1.21	34.55±1.51 b**c**
Maternal age (years)	27.5±5.56	25.0±4.12	30.33±7.48
Cigarettes	1 (10)	1 (14.3)	1 (11.1)
Anesthesia:			
Epidural	3 (30)	4 (57.1)	5 (55.5)
General	0	1 (14.3)	1 (11.1)
Cervical ripening agent:			
None	10 (100)	-	-
Mechanical	-	1 (14.3)	1 (11.1)
Labor, yes	8 (80)	5 (71.4)	3 (33.3)
Labor, hours	7.04±0.25	5.77±4.22	2.22±3.39 b**
Delivery mode:			
C-section, repeat, no labor	-	-	2 (22.2)
C-section, repeat, with labor	-	-	-
C-section, primary, no labor	1 (10)	2 (28.6)	3 (33.3)
C-section, primary, with labor	1 (10)	1 (14.3)	1 (11.1)
Maternal Oxygen given at delivery	2 (20)	3 (42.9)	6 (66.7)
Birth weight (grams)	3321±292.5	2484.3±394.8	1955.6±558.9 a** b**
Placental weight (grams)	484.2±80.46	421.1±134.8	347.8±68.9 b**
Baby's sex: Female / Male	6 (60) / 4 (40)	4 (57.2) / 3 (42.9)	8 (88.9) / 1 (11.1)
Delivery to processing (min)	7.6±16.3	16.1±20.6	29.1±22.3 b*

FGR: fetal growth restriction

a – comparisons were performed between columns with women with physiological pregnancy and the group of women whose pregnancies were complicated by FGR and resulted in term births.

b – comparisons were performed between columns with women with physiological pregnancy and the group of women whose pregnancies were complicated by FGR and resulted in preterm births.

c – comparisons the group of women whose pregnancies were complicated by FGR and resulted in term births with the group of women whose pregnancies were complicated by FGR and resulted in preterm births.

*p<0.05 **p<0.001

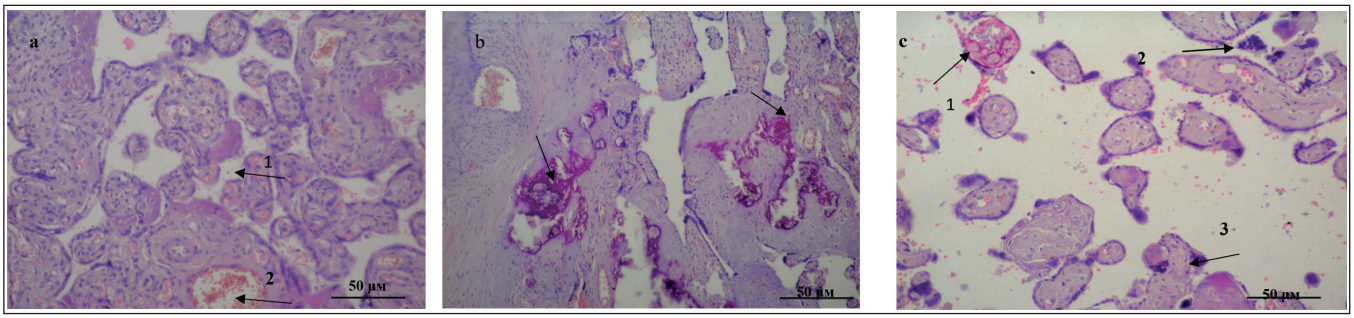

Fig. 1. (a) Terminal, intermediate and stem villi with moderate vascular blood filling, areas of fibrinoid deposition (1) in the intervillous space, general pattern of vascular congestion (2) in women with physiological pregnancies. (b) Areas of deposition of calcium salts in the stroma of the villous chorion of the placentas in the second group of women whose pregnancies were complicated by FGR and resulted in term births. Proliferation of connective tissue in the villi and obliteration of the vascular bed. (c) Avascular villi, areas of reduction of the vascular bed (1) of the placentas in women whose pregnancies were complicated by FGR and resulted in preterm births. Proliferation of syncytium with the formation of symplastic nodules (2) Areas of dystrophic calcification in the terminal villi (3). Staining with hematoxylin and eosin, magn. x200.

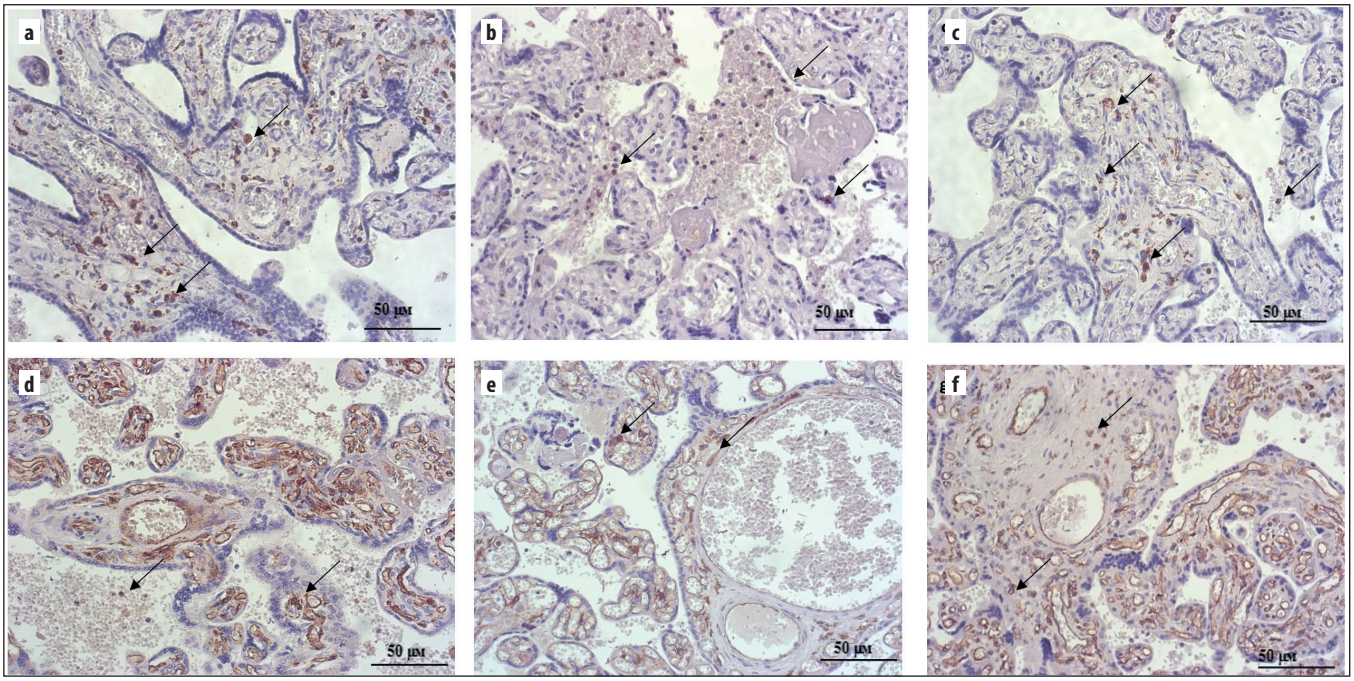

Fig. 2. (a) Expression of CD68 in women with physiological pregnancies; (b) Expression of CD68 in the placentas of women whose pregnancies were complicated by FGR and resulted in term births; (c) Expression of CD68 in the placentas of women whose pregnancies were complicated by FGR and resulted in preterm births; (d) Expression of CD163 in the placentas of women with physiological pregnancies; (e) Expression of CD163 in the placentas of women whose pregnancies were complicated by FGR and resulted in term births; (f) Expression of CD163 in the placentas of women whose pregnancies were complicated by FGR and resulted in preterm births; staining with hematoxylin, magn. x200.

underwent prenatal ultrasound examination starting from 24 weeks of pregnancy. The main criteria for inclusion in the study were as follows: pregnant women, antenatally diagnosed with FGR by ultrasound, namely, the decreased biparietal size of the fetal head, abdominal diameter, hip length and the discrepancy between these sizes and gestational age. The exclusion criteria embraced: patients with pre-existing hypertension, renal failure, diabetes mellitus, gestational diabetes.

The morphological material was fixed in 10% neutral buffered formalin, dehydrated in alcohols and embedded in paraffin. For histological verification, 6-9 pieces were excised from the organ (central, paracentral, peripheral parts of the placenta). To study the structure of the placenta, histological preparations were stained with hematoxylin-eosin. The sections were examined under microscope followed by photographing (x200, x400; Olympus BX-41, Olympus, Germany).

The expression of CD68+ and CD163+ macrophages was investigated in all samples by using immunohistochemical streptavidin peroxidase method. Paraffin sections, 4 μm thick, were deparaffinized and dehydrated, antigens were recovered in citrate buffer in the microwave oven, and endogenous peroxidase was blocked. Further, the sections were incubated at 4°C overnight with murine monoclonal antibodies anti-CD68 (1:25, clone PG-M1, REF PD

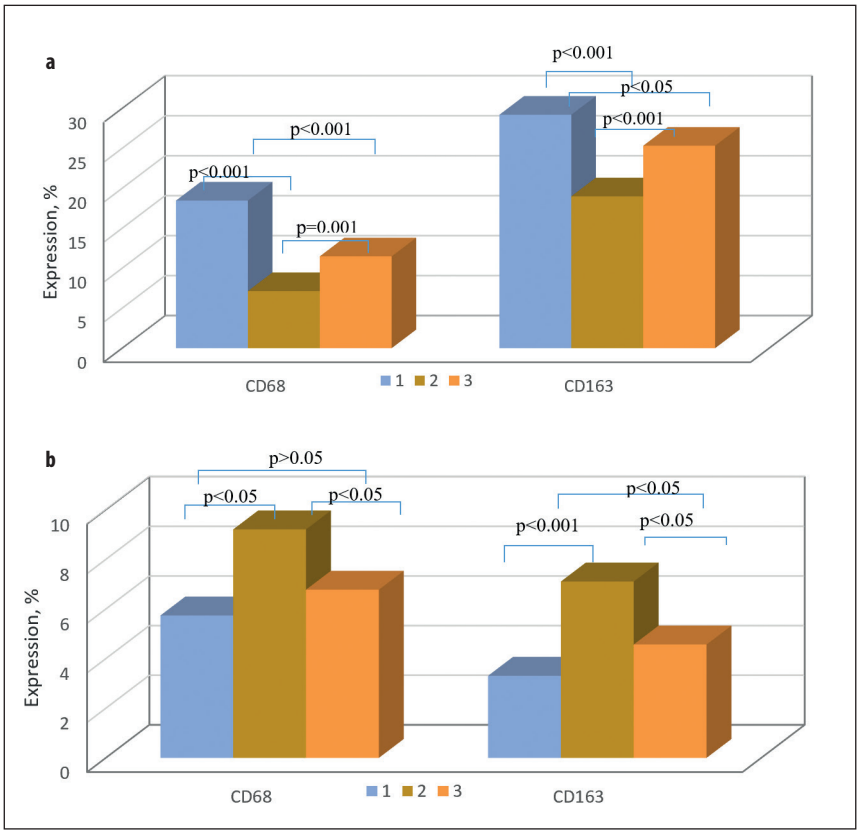

Fig. 3. (a) Indicators of the expression level of the CD68 and CD163 macrophages in the stroma of chorionic villi in women with physiological pregnancies (1), women whose pregnancies were complicated by FGR and resulted in term births (2), and women whose pregnancies were complicated by FGR and resulted in preterm births (3). (b) Indicators of the expression level of the CD68 and CD163 monocytes in the intervillous space of the chorionic villi in women with physiological pregnancies (1), women whose pregnancies were complicated by FGR and resulted in term births (2), and women whose pregnancies were complicated by FGR and resulted in preterm births (3).

M065-S, Diagnostic BioSystems, USA) and anti-CD163 (1:100, clone 10D6, REF Mob460-01, Diagnostic BioSystems, USA). Afterwards, the sections were treated in two steps with the Mouse/Rabbit PolyVue™ HRP/DAB Detection System (Diagnostic BioSystems, USA), with visualization by chromogen; the nuclei were counterstained with Mayer's haemalaun. We used Antibody Diluent buffer as a negative control instead of primary antibodies, and lymph node tissues were used as a positive control. Quantitative indicators were obtained by counting immunopositive $CD68^+$ and $CD163^+$ cells over the entire field of view with a large magnification lens ×40 (high power field, HPF) of placenta. We took into account all obtained quantitative individual data from all fields of view with calculating the mean value. The sections were examined under microscope followed by photographing (x200, x400; Axio Lab. A1, Zeiss, Germany)

Analyses were performed using Prism 5.0 (GraphPad, CA, USA). P-values <0.05 were considered to indicate statistical significance. Normally distributed data were reported using the means with standard deviations, categorical variables were reported using counts and proportions. Comparisons between groups were performed using parametric T-test and nonparametric methods: χ^2 Fischer exact test, Spearman´s correlation test.

RESULTS

The study involved women aged from 18 to 38 years (Table 1). The age of women 1-st group ranged from 19 to 31 years. The age of women 2-nd group ranged from 18 to 38 years. The age of women in the control group – from 22 to 30 years. There were no significant differences in age between groups of women.

The analysis of the number of pregnancies and deliveries indicates that, the majority of women in all groups had their first pregnancy and delivery: among women 1-st group – 4 subjects (57.1%) and 6 subjects (85.7%), respectively; among women 2-nd group – 4 subjects (44.4%) and 5 subjects (55.5%), respectively; among women of the control group – 7 subjects (70%) and 7 subjects (70%), respectively.

The gestational age at which labor developed was reliably shorter in women with FGR, whose pregnancies resulted in both1-st and 2-nd groups, in contrast to women of the control group (p <0.001, p <0.001, respectively). It was found that the duration of labor was longer (by 3.8 times) in women 2-nd group, than in women of the control group (p <0.05).

The parameters of the body weight of newborns were significantly lower among women both 1-st and 2-nd groups, than among women of the control group (p <0.001, p <0.001, respectively). The placenta weight was lower (by 1.7 times) in women 2-nd group, than in women of the control group (p <0.001).

Histological examination of the placentas of women both1-st and 2-nd groups, in contrast to women in the control group, showed a discrepancy between its maturity and gestational age, and the disrupted maturation of chorionic villi, predominantly of the dissociative type (Fig. 1). Thus, among the mature terminal villi in the specimens of this group, were found both mature and immature intermediate villi, groups of chaotic sclerosed villi. Quite often, zones of

involutive and dystrophic processes were registered in the form of afunctional areas, in particular, due to excessive deposition of fibrinoid in the intervillous space. In women of control group fibrinoid deposits were diffusely focal, mainly in the peripheral parts of the placenta, which indicated its disrupted maturation and inflammatory changes.

Much more often, was observed significant areas of dystrophic calcification in the placentas of women 1-st group, in contrast to women 2-nd group (5 subjects; 71.5% versus 1 subject, 11.11%; $p < 0.05$). In the placentas of women 2-nd group, changes in the stroma of the villi were noted, which were characterized by an increase in the connective tissue, increased collagen formation, foci of necrosis, an increase in calcification deposits in syncytial nodules, which practically excluded their role in the adaptation process of villi.

In the placenta of women 2-nd group, more pronounced signs of impaired vascularization (hypovascularization) and hypoplasia of chorionic villi were manifested, which indicated the inability of terminal villi to carry out compensatory reactions to the full extent. In particular, the proliferation of terminal villi in the placentas of women 2-nd group was much less frequently determined, in contrast to women in the control group (1 subject, 11.1% versus 7 subjects, 70%; $p < 0.01$). At the same time, destructive and degenerative processes were combined with signs of compensatory and adaptive reactions in both groups of women, whose pregnancies were complicated by FGR.

In the placentas of women of the control group, were observed a sufficient level of compensatory and adaptive reactions in terminal villi with the formation of syncytial nodules, capillary hyperplasia, and active formation of syncytiocapillary membranes.

Expression of CD68 marker (Fig. 2, 3) was found in the cytoplasm of macrophages located in the stroma of intermediate and terminal villi, as well as in monocytes of the intervillous space. The level of CD68+ macrophages in terminal villi of the placenta was significantly lower in women both 1-st and 2-nd groups than in women of the control group ($p < 0.001$; $p < 0.001$, respectively). The level of CD68+ macrophages in the placentas of women 2-nd group was significantly higher than in placentas of women 1-st group ($p < 0.001$). The level of CD68+ monocytes in the intervillous space of the placenta was significantly higher in women 1-st group as compared to women of the control group ($p < 0.05$) and women 2-nd group ($p < 0.05$).

Fig. 3. (a) Indicators of the expression level of the CD68 and CD163 macrophages in the stroma of chorionic villi in women with physiological pregnancies (1), women whose pregnancies were complicated by FGR and resulted in term births (2), and women whose pregnancies were complicated by FGR and resulted in preterm births (3). (b) Indicators of the expression level of the CD68 and CD163 monocytes in the intervillous space of the chorionic villi in women with physiological pregnancies (1), women whose pregnancies were complicated by FGR and resulted in term births (2), and women whose pregnancies were complicated by FGR and resulted in preterm births (3).

Expression of CD163 marker was registered mainly on the membrane of macrophages, which were found in the stroma of terminal villi, as well as on the surface of monocytes localized in the intervillous space. The level of CD163+ macrophages in terminal villi of the placenta was significantly lower in women both 1-st and 2-nd groups, in contrast to women of the control group ($p < 0.001$, $p < 0.001$, respectively). The level of CD163+ macrophages in this compartment of the placenta was significantly lower in women 1-st group as compared to women 2-nd group ($p < 0.001$). The level of CD163+ monocytes in the intervillous space of the placenta is significantly higher in women 1-st group as compared to women of the control group ($p < 0.001$) and women 2-nd group ($p < 0.05$). A significantly higher level of CD163+ monocytes in the intervillous space was revealed in women 2-nd group as compared to women in the control group ($p < 0.05$).

Analysis of the expression ratio of CD68+ / CD163+ macrophages subpopulations demonstrated that in the stroma of terminal villi the M2 profile significantly predominates over M1 in the placentas of women of the control group ($p < 0.001$), as well as in women 1-st group ($p < 0.001$) or 2-nd group ($p < 0.001$). Analysis of the expression ratio of CD68+ / CD163+ monocytes subpopulations demonstrated that in the intervillous space, the M1 pro-inflammatory profile exceeds the M2 anti-inflammatory profile in the placentas of women of the control group ($p < 0.05$), as well as women 2-nd group ($p < 0.001$).

In women of the control group, correlation analysis revealed a significant relationship between the expression levels of CD68+ and CD163+ macrophages in the stroma of terminal villi of the placenta ($r = -0.787$; $p = 0.007$), and in women 2-nd group – between the levels of CD68+ monocytes in the intervillous space and the weight of a newborn ($r = -0.765$; $p = 0.016$).

DISCUSSION

In the current study it was shown that in women 2-nd group was lower indicators of the placenta weight; they gave birth at an earlier gestational age and after a prolonged labor period, in contrast to women 1-st group or women control group. Our findings are consistent with studies that investigated the relationship between the postpartum placental morphometry and infant birth weight. It has been shown that low birth weight is comparable to low parameters of weight, volume and area of the placenta in FGR [10]. As our results have shown, in FGR, there are characteristic signs of placental insufficiency. Vascularization, development and growth of villi are particularly important morphological indicators of the state of the placenta. Meanwhile, in the placentas of women 1-st group, we identified the morphological changes that indicate the presence of a state of hyperfunction with inclusion of compensatory and adaptive mechanisms. At the same time, the changes in the placentas of women 2-nd group indicate the insufficiency of compensatory processes, manifested by the disrupted vascular and stromal component of the villous chorion and the development of involutive and

dystrophic changes. It has been suggested that morphological changes in the placenta in FGR may be caused by placental perfusion due to a decrease in the vascular bed and hypovascularization of terminal villi [11].

Macrophages (Hofbauer cells) play a crucial role in pregnancy, and their dysfunction or alteration of polarity is involved in pregnancy disorders, like FGR and preterm labor. We have demonstrated that macrophages with CD68+ and CD163+ phenotypes are detected in all groups of placentas. It is likely that macrophages are influenced by factors of the local microenvironment, which contributes to polarization, taking into account the conditions in the placenta during pregnancy. However, one cannot exclude the influence of macrophages themselves on a number of processes in the placenta, including their control over the development and remodeling of chorionic villi; control over angiogenesis of villi; control of trophoblast transformation and the formation of syncytial nodules [12].

Our data on the prevalence of CD163+ M2 macrophages population over CD68+ phenotype of M1 macrophages in the stroma of terminal villi of the placenta in all groups of women are partially consistent with other studies, which assume that CD163+ macrophages (Hofbauer cells) are constitutively expressed in all compartments of the placenta throughout pregnancy, whereas CD68+ macrophages have variable expression over time, and its intensity peaks in the second trimester and decreases as pregnancy progresses [13,14]. At the same time, it is important to note that the functions of M1 and M2 macrophages in the placenta in FGR remain completely unclear and contradictory.

It was found a more pronounced increase in the number of CD68+ macrophages in the placenta of women 2-nd group, in contrast to the indicators of women 1-st group, and these findings are consistent with the results of other authors. It is likely that the increased level of the pro-inflammatory profile of CD68+ macrophages in preterm labor can be explained by the participation of these cells in several processes (maturation of the fetoplacental tissue, preparation of the uterus for childbirth, etc.), which are accompanied by activation of inflammatory processes [15]. The revealed overexpression of CD68+ pro-inflammatory monocytes in women whose pregnancies resulted in preterm births is consistent with the findings of several authors [16], indicating that peripheral blood mononuclear cells entering the intervillous space can begin to differentiate into mature M1 macrophages under the influence of M1-activating inflammatory environmental stimuli. However, if necessary, they can repolarize to M0 or even M2 macrophages, depending on the needs and influence of the microenvironmental factors [17].

CONCLUSIONS

Thus, in FGR, the placenta undergoes the development of pathomorphological changes characteristic of placental insufficiency. In the present study, in the placentas of women whose pregnancies were complicated by FGR and resulted in term births underwent transformation of the chorionic villi and the vascular component, a decrease in the number of CD68+ M1 profile macrophages with a predominance of CD163+ M2 macrophages population of the placenta as opposed to physiological pregnancies. Therefore, one can observe the disruptions of compensatory mechanisms with their partial adaptation, since CD68+ macrophages of the pro-inflammatory pool are directly associated with inflammatory infiltration of the placenta and an increase in the proliferation of terminal villi with compensation of metabolic processes, whereas CD163+ macrophages of the anti-inflammatory pool affect the formation of hemodynamic adaptive reactions.

Finally, in the placentas of women whose pregnancies were complicated by FGR and resulted in preterm births, there were disruptions of the vascular and stromal component of the chorionic villus with the development of involutive and dystrophic changes due to a decrease in the average area of terminal villi. In this case, the role of macrophages should be considered in a much broader aspect. In our opinion, their participation is associated with a disruption of compensatory mechanisms in the placenta, since the number of the CD68+ macrophages of the pro-inflammatory pool is negatively associated with inflammatory infiltration of the placenta. This is presumably due to dysregulatory processes caused by hypovascularization that is also associated with a reduced birth weight, which generally may determine the development of chronic placental insufficiency and enhance the formation of FGR. This results will contribute to improve the prognosis and diagnosis of FGR and fetal prematurity.

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ORIGINAL ARTICLE

SIMULTANEOUS OPERATIONS DURING UMBILICAL AND PARAUMBILICAL HERNIA REPAIR: POSSIBLE OR NECESSARY?

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ABSTRACT

The aim of the study was to determine the possibility and effectiveness of simultaneous surgical interventions in umbilical and paraumbilical hernia repair.

Material and methods: 148 case histories were analyzed concerning patients who were routinely admitted to the surgical department of the Kharkiv Regional Council's Municipal Non-Profit Enterprise «Regional Clinical Hospital» between 2017 and 2019, and who underwent umbilical and paraumbilical hernia repair simultaneously with operations related to some other surgical pathology (group 1, n = 67) or in separate interventions (group 2, n = 81). All patients were routinely operated after a set of mandatory and additional general clinical, laboratory and instrumental research conducted in accordance with the existing guidelines. The structure and results of surgical interventions related to the underlying disease and simultaneous operations were studied.

Results: Simultaneous operations were performed for comorbid cholecystolithiasis, diaphragmatic esophageal hernia with gastroesophageal reflux, inguinal hernia, white line hernia, benign diseases of the uterus and uterine appendages et al. The frequency of complications and recurrences of hernia in patients with simultaneous and isolated of umbilical hernia repair did not differ significantly. The outcome of the operation mostly depended on the method of operation (postoperative complications were most often observed in open sutures repair and were absent in laparoscopic hernia repair). Additional risk factors were weight gain and diabetes.

Conclusions: Summarizing the data obtained, it can be concluded that application of modern endovideoscopic techniques in surgery makes simultaneous surgical interventions not only possible but also necessary in the presence of concomitant abdominal pathology that requires surgical treatment.

KEY WORDS: mbilical hernias, paraumbilical hernias, simultaneous operations, postoperative complications, hernia recurrences

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INTRODUCTION

In most countries of the world, as far as the structure of routine and emergency abdominal interventions is concerned, hernia repair occupies one of the leading places. According to a population-based study, the anterior abdominal wall hernias in patients older than 10 years of age are found in 20.9% of the population, and most often it is umbilical hernia, with prevalence of 10.2% [1, 2]. Based upon the results of their ultrasound studies, M.A. Bedewi et al. established that prevalence of umbilical and paraumbilical hernias can be as high as 25% [3]. Among the patients who underwent surgery the incidence rate of such hernias is 13.3% [4].

The optimal method of treatment of patients with umbilical and paraumbilical hernias is considered to be the one that involves open access mesh endoprosthesis hernia repair with peritoneal mesh placement. Laparoscopic access may be useful in cases of a large hernia defect and an increased risk of wound complications [5]. However, in recent years there have been numerous reports concerning simultaneous, or concurrent, surgical treatment of two or more abdominal diseases that require surgical intervention [6-9].

In terms of the impact of access and plastics method on immediate and long-term results of hernioplasty, the choice of hernia repair method in simultaneous operations remains pertinent and requires further research.

THE AIM

The aim of the study was to determine the possibility and effectiveness of simultaneous surgical interventions in umbilical and paraumbilical hernia repair.

MATERIAL AND METHODS

148 case histories were analyzed concerning patients who were routinely admitted to the surgical department of the Kharkiv Regional Council's Municipal Non-Profit Enterprise «Regional Clinical Hospital» between 2017 and 2019, and who underwent umbilical and paraumbilical hernia repair simultaneously with operations related to some other surgical pathology (group 1, n = 67) or in separate interventions (group 2, n = 81).

Table 1. Concomitant pathology in patients of group 1 and 2.

Descriptor	Group 1	Group 2	p
Gender, F/M	40 (60%)/ 27 (40%)	36 (44%)/45 (55%)	>0.05[1]
Average age, years	51.3±13.2	51.3±16.1	>0.05[2]
Type of hernia: - umbilical - paraumbilical	 65 (97%) 2 (3%)	 73 (90%) 8 (9.9%)	>0.05[2]
Size of hernia: - < 2 cm - 2-4 cm > 4 cm	 13 (19%) 41 (61%) 13 (19%)	 14 (17%) 53 (65%) 14 (17%)	>0.05[2]
Concomitant pathology:			
Diabetes mellitus, n (%)	4 (6%)	4 (5%)	>0.05[1]
Excess weight, n (%): Body mass index 25-30 kg/m^2 Body mass index > 30 kg/m^2	 22 (33%) 7 (10%)	 24 (30%) 4 (5%)	>0.05[1]
Arterial hypertension, n (%)	29 (43%)	27 (33%)	>0.05[1]
Coronary heart disease, n (%)	7 (10%)	11 (14%)	>0,05[1]
Chronic cardiac failure, n (%)	12 (18%)	12 (15%)	>0.05[1]
Chronic obstructive pulmonary disease, n (%)	2 (3%)	4 (5%)	>0.05[1]

Note: [1] reliability in regard to χ2 criterion; [2] reliability in regard to t-test

Table 2. Methods of umbilical hernia repair surgery.

Descriptor	Group 1	Group 2
Laparoscopic alloplasty (IPOM technique)	40 (60%)	16 (20%)
Hybrid alloplasty	23 (34%)	–
Open alloplasty:		
Onlay technique	–	2 (2.5%)
Inlay technique	1 (1.5%)	8 (10%)
Sublay technique	2 (3.0%)	37 (46%)
Mayo open autografting	1 (1.5%)	18 (22%)

Note: IPOM – intraperitoneal onlay mesh.

The patients in both groups were similar as far gender, age and frequency of comorbidities (Table 1). Various methods of hernia repair surgery were used for treatment of umbilical hernias (Table 2).

IPOM hernia repair and hybrid alloplasty predominated in patients who underwent simultaneous surgeries (group 1): in a number of cases open hernia repair techniques were used. In group 2 mesh endoprosthesis alloplasty was generally performed, and open Mayo hernia repair was performed quite often.

In addition to the above mentioned concomitant pathology, the patients of group I had other diseases, which were indications for simultaneous surgery (table 3).

All patients were routinely operated after a set of mandatory and additional general clinical, laboratory and instrumental research conducted in accordance with the existing guidelines. The structure and results of surgical interventions related to the underlying disease and simultaneous operations were studied.

The obtained results were processed using PSSP statistical software package and applying the frequency analysis method and making use of Fisher's Exact Test and χ2 criterion for comparison of qualitative characteristics, and the t-test for comparison of quantitative characteristics. The difference between the groups was considered to be significant at ($p<0.05$). The results are given in the form of absolute count (%) for qualitative characteristics and in the form of M±SD (mean and standard deviation of the mean) for quantitative characteristics.

RESULTS

In the early postoperative period 21 (14.2%) complications were detected; usually it was a seroma and hematoma in the postoperative wound, less frequently it was infiltrate inside the wound, which did not require additional interventions. In one case sutures had to be removed due to surface inflammation in a limited area. Recurrences were detected in 9 (6.1%) patients. No impact of simultaneous surgery on the development of complications, duration of hospitalization and recurrence rate one year after surgery and

Table 3. Comorbidities and simultaneous operations in regard to Group I patients

Diagnosis	Operation	Number	%
Cholecystolithiasis	Laparoscopic cholecystectomy	30	45
Hernia of the esophageal orifice of the diaphragm	Posterior crurorаphia, Nissen fundoplication	6	9
Inguinal hernia	TAPP hernia repair	4	6
Epigastric hernia	IPOM hernia repair	1	1.5
Benign diseases of uterus	Uterectomy	12	18
Adnexal diseases	Salpingectomy / ovariectomy, cystectomy	8	12
Adiposity	Sleeve Gastrectomy	3	4.5
Benign skin tumors	Tumor removal	1	1.5
Urachal cyst	Urachal cyst removal	1	1.5
Chronic appendicitis	Laparoscopicappendectomy	1	1.5

Note: Transabdominal pre-peritoneal – TAPP; Transabdominal pre-peritoneal (TAPP).

Table 4. Actual results of operations.

Descriptor	Group 1	Group 2	p
Wound complications, n (%):	8 (12%)	13 (16%)	
- seroma	4 (6%)	5 (6%)	
- hematoma	3 (4.5%)	5 (6%)	>0.05[1]
- infiltrate	1 (1.5%)	2 (2.5%)	
- inflammation	–	1 (1%)	
Duration of hospitalization, days	6.7±1.5	6.5±2.0	>0.05[2]
Recurrences, n (%)	3 (4.5%)	6 (7%)	>0.05[1]

Note: [1] reliability in regard to χ2 criterion, [2] reliability in regard to t-criterion;

Table 5. Incidence of complications and recurrences, depending on the hernia repair techniques

Surgical technique	Complications	Hernia recurrence
Mayo open hernioplasty, n=19	5 (26%)	3 (16%)
Open alloplasty, n=50	8 (16%)	4 (8%)
Laparoscopic alloplasty (IPOM technique), n=56	3 (5%)	–
Hybrid alloplasty, n=5	5 (22%)	2 (9%)

Table 6. Incidence of complications and recurrences depending on the size of hernia and concomitant diseases, not related to abdominal cavity.

Descriptor	Complication	Hernia recurrence
Size of hernia:		
< 2 cm, n=27	4 (15%)[1]	1 (4%)[1]
2-4 cm, n=94	12 (13%)	4 (4%)
> 4 cm, n=27	5 (18,5%)	4 (15%)
Excess weight (Body mass index over 25 kg/m^2):		
yes, n=57	12 (21%)[1]	8 (14%)[2]
no, n=91	9 (10%)	1 (1%)
Diabetes mellitus:		
yes, n=9	2 (22%)[1]	4 (44%)[2]
no, n=139	19 (14%)	5 (4%)

Note: [1] p>0.05 in regard to χ2criterion, [2] p<0.05 in regard to χ2criterion.

later was detected one year after surgery and later. On the contrary, the incidence of complications and recurrences in group 1 demonstrates a tendency to reduction (table 4).

This tendency can be explained by the difference in the structure of hernioplasty techniques used for group 1 and group 2, which is demonstrated by the analysis of actual results, depending on umbilical and paraumbilical hernia repair techniques utilized (table 5).

The greatest number of complications was observed following open hernia repair, which was generally used for group 2 patients, and the smallest number of complications was reported after laparoscopic alloplasty, which

was prevalent in patients who underwent simultaneous operations (group I).

Incidence of complications and recurrences depending on the presence of known risk factors was analyzed separately (table 6).

An unsubstantiated tendency to increase of the number of complications with the increase in hernia size, weight gain and in the presence of diabetes was identified. More pronounced relationships can be determined by analyzing the incidence of recurrences: the latter most certainly increased with the increase in weight and in the presence of diabetes mellitus.

DISCUSSION

Comparison of the incidences of complications after simultaneous and separate umbilical hernia repairs did not show significant differences (12% and 16% respectively, with p> 0.05). In both groups, the most common were seromas, which were found in 6% of cases in each group, and hematomas – 4.5% and 6% respectively (p> 0.05). In several cases infiltrate or inflammation of the postoperative wound were observed. This is consistent with the results obtained by other researchers. Thus, Shankar et al. observed wound complications in 18% of cases, among which percentage seromas amounted to 6.3% [10]. But in a study by M.W. Christoffersen et al. the incidence of seromas after IPOM hernia repair amounted to 58% (in regard to large- and medium-sized umbilical hernias) [11], while J.M. Shao et al. reported a much lower incidence of complications: surgical site infection – 2.7%, hematomas – 1.1%, seromas – 2.7% [12]. This scatter is likely due to additional factors that need to be considered when assessing the development of complications. It should be noted that there were no differences between the groups in terms of gender, age, frequency of concomitant non-surgical pathology and characteristics of hernia.

According to our findings, the method of surgery was the most influential factor in the development of complications. Thus, after using conventional open suturing technique with tissue materials (Mayo) the incidence of complications amounted to 26%; the lowest incidence was observed after laparoscopic alloplasty – 5%. However, quite high incidence of complications was also observed after open mesh alloplasty (16%) and hybrid alloplasty (22%), and most of complications were represented by seromas and hematomas. More frequent formation of seromas and hematomas following the use of such hernia repair techniques can be explained by an enlarged wound, the need for tissue separation manipulations and the presence of a mesh endoprosthesis.

Similar tendencies were established when comparing the incidence of recurrences. In the group of patients who underwent simultaneous surgical operations, during observation lasting from 1 to 2 years they were observed in 4.5% of the cases, and in the group of patients who underwent separate hernia repair – in 7% of the cases (p>0.05). This is also consistent with the results obtained by other researchers. Thus, K. Donovan et al. found that the umbilical hernia recurrence rate after open mesh or suture hernia repair amounted to 3.3% [13]. According to M.W. Christoffersen et al., even for small-sized hernias the recurrence rate is as high as 14% in 3 years following the surgery [14]. Shankar et al. reported development of recurrences within 3.1 years after surgery on average in 6% of patients [10].

To a large extent it also depends on the method of surgery. In our study there were no recurrences after IPOM hernioplasty; hernia recurrence was most commonly encountered after open suture autografting (Mayo technique) – 16%, after open and hybrid autografting – 8% and 9% respectively. Similar results were obtained by other authors: the recurrence rate after using suture techniqus amounted to 9.8%, after using mesh herniopasty – to 2.4% [10]. In a randomized, double-blind, controlled, multicenter study R. Kaufmann et al. found that within 30 months following the operation the recurrence rate after mesh hernia repair of umbilical hernias from 1 to 4 cm in size amounted to 4%, after suture hernia repair – to 12%. The most common complications were seromas, hematomas, and wound infections (1-2%) [15].

In addition to specificity of the surgical intervention, the incidence of complications and recurrences depended on other factors. In particular, a tendency to increase in their number when dealing with hernias larger than 2 cm in size was detected in overweight patients and in the presence of diabetes mellitus. With this, it can be claimed with a high degree of certainty that the incidence of recurrences really increased. These factors are considered to be the hernia recurrence risk factors, including simultaneous laparoscopic inguinal hernioplasty, smoking, open suture hernia repair of hernias over 1.5 cm in size and inflammation in the wound area, ascites and liver diseases [10].

The results of our research demonstrate that additional simultaneous surgery does not add to the risk of complications and recurrences. However, in order to improve the results of umbilical hernia surgery, in each particular case it is necessary to carefully select the best method of hernioplasty. First of all, it should be noted that due to the high incidence of complications and recurrences the conventional open suturing technique with tissue materials is not the best choice. Although some authors believe that the choice between alloplasty and autografting is often based on the size of hernia: if the size of hernia gate is less than 2.0 cm (and in the opinion of Z.Tao et al. [16] less than 2.3 cm), autografting can be a possible choice, and if said size is larger, mesh graft alloplasty should be used. But we believe that in addition to this factor it is necessary to take into consideration the thickening of the abdominal wall, which is observed in obese patients. In such cases we recommend to use alloplasty for repair of even small-sized umbilical hernias. We applied this method mainly in the beginning of the reporting period, predominantly for treatment of small hernias. However, even when treating small hernias (<2.0 cm in size) in overweight patients this method can cause complications and lead to recurrences.

Due to proper planning of simultaneous operations in our study, they were conducted using the laparoscopic method, which enables additional intervention without additional trau-

matizing effect. Also of interest is the hybrid approach, which involves elements of open umbilical hernia repair and has the advantages of laparoscopic imaging. This approach has been used by many researchers. Thus, F.P. Prete et al. used laparoscopic imaging during open umbilical hernia repair to monitor placement of mesh endoprosthesis on the peritoneum and to re-position it in 14.3% of the cases [17]. J.M. Shao et al. also used a hybrid approach – open laparoscopic technique – and additionally performed transabdominal inguinal hernia repair in 58% of the patients [12]. We believe that in patients who are to undergo simultaneous surgery in the upper abdominal cavity (in particular, for cholecystolithiasis and/or hiatal hernia), in the presence of a small hernia (less than 2 cm in size) it is highly recommended to use hybrid method of umbilical hernia repair – open access through an incision for a trocar used for laparoscopic cholecystostomy with mesh hernia repair and laparoscopic imaging. For medium (2-4 cm) and large (over 6 cm) hernias and in the presence of obesity and multiple hernias, regardless of the size of the hernia gate, the best choice is IPOM hernia repair using a mesh with anti-adhesive coating, larger than the size of the hernia gate by 5 cm or more.

CONCLUSIONS

Summarizing the data obtained, it can be concluded that application of modern endovideoscopic techniques in surgery makes simultaneous surgical interventions not only possible but also necessary in the presence of concomitant abdominal pathology that requires surgical treatment.

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ORIGINAL ARTICLE

LIFE QUALITY OF EMPLOYEES OF THE NORTH-EASTERN FEDERAL UNIVERSITY (YAKUTSK)

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ABSTRACT

The aim of the study evaluate the quality of life of employees of the North Eastern Federal University.

Materials and methods: The respondents to the WHO QoL 100 questionnaire were 37 teachers and 32 support staff with an average age of 44 years.

Results and conclusions: There is a higher correlation between assessments of overall satisfaction with the quality of life and individual areas in the group of surveyed teachers compared to the group of support staff. Comparison of the parameters of the QOL areas of teachers of NEFU with the data of similar surveys of universities in Central Russia revealed a higher assessment of such areas as the "environment", the "physical" sphere among the Yakutia's. These results indicate the adaptability of NEFU employees to environmental conditions. Also, among the respondents of the Yakutia's, there was revealed a great significance for the QOL of such a subsphere as support for relatives, friends and colleagues.

KEY WORDS: quality of life, WHO QoL-100 questionnaire, university staff, Yakutia

Wiad Lek. 2021;74(2):225-227

INTRODUCTION

Production of natural resources in the North is possible if people will settle there, which requires the improvement of the socio-economic living conditions and improving the quality of life (QoL). During 1991-2015 Yakutia lost almost 300 thousand people because of migration outflow [1]. The world health organization proposed to consider the quality of life as "the individual perception of his position in life in the context of the cultural environment and value system in which the individual lives, and in relation to his goals, expectations, standards and beliefs" [2]. The study of QoL of University teachers in Central Russia using international questionnaires WHOQ QoL-100, SV-36 was carried out in a number of papers [3, 4]. However, the study of QoL in employees of the University, located in a region with uncomfortable natural conditions is of particular interest.

THE AIM

The aim of the study evaluate the quality of life of employees of the North Eastern Federal University.

MATERIALS AND METHODS

To study the quality of life, the basic questionnaire of quality of life ("WHOQ QoL-100") adapted for Russia at the research Institute of V. M. Bekhterev was used. The questionnaire includes 100 questions, and is a subjective scale (in points), designed to be filled by the Respondent. The scale of the questionnaire allows to assess the quality of 6 spheres of life: physical, psychological, spiritual sphere, the level of independence, social relationships and the environment. Each of the 6 spheres consists of subspheres, their total number is 24. The quantitative characteristic of the total QoL is the sum of the values of all six spheres (the maximum value is 104.5 points). At the end of the questionnaire four global questions are included (G1-G4), the total score of answers to which (G) allows you to give an integral assessment of the Respondent's satisfaction with the quality of life and health. Reduction of parameters of QoL testifies decreasing of general state of health and adaptive possibilities of an organism.

Two groups of female Yakut employees of NEFU were interviewed: the first group consisted of 37 people from the University professorial teaching staff (PTS) and the second group-32 people from the University training and support staff (TSS). Information was collected based on informed consent of respondents in compliance with the principles of bioethics. The average age of the University professorial teaching staff was 45.4 years, the University training and support staff was 42.4 years.

Statistical processing included the analysis of distributions, their proximity to the standard, finding the average values, the standard error of the average. The significance of the differences between the data of different groups was assessed using the Student's T-test. A correlation analysis of the relationship between the studied parameters was carried out.

RESULTS AND DISCUSSION

Differences of values of parameters of spheres of QoL between PTS and TSS are revealed (table 1). In the phys-

Table I. Results of QoL spheres in points (M±m) NEFU respondents

Sphere	UTS	USS	P
Phisical	14.00±0.43	12.82±0.43	0.047
Psychological	14.40±0,41	12.55±0.45	0.003
Level of independence	16.13±0.66	15.78±0.38	0.706
Social relationships	15.22±0.48	14.20±0.46	0.140
Environment	13.26±0.32	11.91±0.30	0.002
Spiritual	15.9±0.62	14.12±0.54	0.026
Total	88.3±2.10	80.5±2.00	0.009

Table II. Correlation coefficients between estimates of satisfaction with quality of life and individual areas of PTS (1) and TSS (2)

The life quality spheres									
Environment		Psychological		Level of independence		Physical		Social relationships	
1	2	1	2	1	2	1	2	1	2
0.85	0.81	0.84	0.63	0.78	0.36	0.69	0.61	0.6	0.59

ical sphere, QoL is significantly higher in the group of professorial teachers mainly due to the better indicator of the subsphere "sleep quality" compared to the training and support staff.

In the psychological sphere as a whole value of indicators at PTS are higher, than at TSS. In the components of this sphere concerning confidence, satisfaction with their abilities revealed a higher self-esteem of intellectual abilities memory, attention and learning. Significantly, higher rates were observed in PTS in the field of "environment". Thus, clearly expressed higher self-esteem in the subsphere of "Opportunities for the acquisition of new information and skills" in PTS (p=0.002) opposite TSS, which may be associated with ongoing training courses for teachers. There is a tendency to a higher assessment of the quality and comfort of housing (p=0.07) in PTS (15.5±0.66) relative to TSS (13.7±0.82). PTS respondents also showed a tendency (p=0.01) to score higher in the "satisfaction with financial situation" sub-sphere (11.0±0.68) compared to TSS (9.64±0.64). Respondents in the PTS group had significantly higher rates in the spiritual sphere than in the TSS group. It is obvious that on the basis of the scientific worldview, PTS has formed more stable ideas about the meaning of life, which allow to resist the current life challenges. The indicator of the overall quality of life, calculated as the sum of the values of all six areas of the questionnaire, is significantly higher among PPS respondents, relative to TSS (table 1). It is necessary to note the large values of the total assessment of QoL in both groups of NEFU employees compared with teachers of the same age of Central Russian universities (74.4) [4].

The greatest differences between the teaching staff of Yakutsk and teachers of medical schools of Central Russia were in the assessment of the sphere "environment", respectively 13.26 and 9.69. The high rating of this sphere of the Yakuts may be due, on the one hand, adaptation to harsh climatic conditions, and the satisfaction with financial situation: payment of the regional factor and Northern extra charges, partially offsetting the uncomfortable living conditions in the North. Also, the Yakuts had a higher assessment of the sphere of "Social relations" (15.22) relative to the teaching staff of universities in Central Russia (13.14). It is obvious that in the harsh natural and climatic conditions of the Yakuts own QoL to a large extent associated with the support of relatives, friends and colleagues.

The study of correlations between the overall assessment of satisfaction with their QoL (G) and indicators of individual areas is of interest. Positive correlations were found in both groups (table 2), which is consistent with the results of the study of QoL of University teachers in Central Russia [4]. The closest positive relationship of subjective assessment of QoL was revealed with the sphere of "environment" in both groups. The close relationship with the parameters of this sphere indicates that the subjective assessment of satisfaction with life expectancy is largely determined by the socio-economic status of respondents: income level, housing conditions, security of residence, medical care. For PTS higher values, in comparison with TSS, have indicators of the psychological sphere, including cognitive processes that allow teachers effectively perceive and process incoming information. PTS also attaches more importance to maintaining the "level of independence" of QoL compared to TSS (table 2). It is obvious that teachers appreciate the role of physical fitness in achieving high performance of their professional activities.

CONCLUSIONS

In conclusion, it should be noted that a comparative analysis of QoL in NEFU employees with different specifics of professional activity revealed a significantly higher final score in PPS relative to TSS. The most significant difference in responses between PPS and TSS was revealed in the areas of "Environment", "Psychological", "Spiritual". These areas were logically justified by higher grades in teaching staff on questions about the role of new information, self-as-

sessment of intellectual abilities, the strength of ideological beliefs, that is, those components of life, which are most important in the professional activity of the teacher. Comparison of the parameters of QoL spheres with the data of teachers of universities of Central Russia [3] revealed a higher assessment of such spheres as "Environment", "Physical" spheres among the Yakuts. These results indicate the adaptation of NEFU PPS to environmental conditions. Also, the Yakut respondents revealed a great importance for the life of such a subsphere as the support of relatives, friends and colleagues.

Despite the difference in the indicators of various spheres of PPS and TSS, the integral subjective assessment of their own quality of life and health between the groups did not differ, which reflects the overall satisfaction with the situation of respondents in the social environment of the region.

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ORIGINAL ARTICLE

MORPHOLOGICAL CHANGES OF THE GASTRIC MUCOSA WHILE USING ANTIAGGREGANTS AND PANTOPRAZOLE (AN EXPERIMENTAL STUDY)

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ABSTRACT

The aim: Was to characterize the morphological peculiarities of the gastric mucosa at early stage of prescription of acetylsalicylic acid (ASA) and clopidogrel as well as to study the impact of pantoprazole on the gastric mucosa to optimize the prophylaxis and treatment of gastropathies induced by ASA and clopidogrel.

Materials and methods: The experiments were performed on 77 non-linear white male rats with the average weight of 150-180 g. Depending on the aim of research, the animals were divided into 7 groups.

Results: The administration of pantoprazole in combination with ASA and clopidogrel presented positive trends in neutral glycoproteins amount and contributes to preventing GM necrotic lesions by amplification of protective properties of mucus and stabilization of apoptotic activity of gastric epithelial cells.

Conclusions: 1. According to our study findings, administration of ASA in combination with clopidogrel results in 2,5 times higher risk of GM erosive lesions.

2. One of the most significant morphological manifestations of gastropathy in ASA and clopidogrel regimen is the development of microerosions, which are poorly diagnosed by macroscopic examination.

3. The use of PAS-reaction makes possible to identify damage to the basal membrane of superficial epitheliocytes, which may be a top-priority morphological criterion of gastropathy induced by ASA or clopidogrel in the absence of an inflammatory reaction.

4. Administration of pantoprazole in combination with ASA and clopidogrel contributes to preventing GM necrotic lesions by amplification of protective properties of mucus and stabilization of apoptotic activity of gastric epithelial cells.

KEY WORDS: acetylsalicylic acid, clopidogrel, pantoprazole, morphological changes, stomach erosive lesions

Wiad Lek. 2021;74(2):228-235

INTRODUCTION

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) remains extremely actual in treating pain, inflammation in antiplatelet therapy. These remedies belong to the preparations most widely used in the practice of doctors of different specialties [1, 2].

The consequence of such a wide, sometimes incontrollable, taking preparations is a high frequency of complications which emerge due to the damage of the gastrointestinal tract (GIT) and got the name of the NSAIDs gastropathies. [3]

One of the most widely spread representatives of inflammatory preparations is acetylsalicylic acid (ASA). ASA used in low (antiplatelet) doses remains by now a rather effective and cheap remedy of the profilaxis of vascular catastrophes linked to IHD and atherosclerosis [4].

At the same time, there exist a number of limitations for the indication of ASA and this is, first of all, the development of erosive ulcerative lesions of the stomach. The risk factors of the emergence of gastropathy are: peptic ulcer of the stomach and duodenum, the age over 60, taking other preparations (glucocorticoids, anticoagulants NSAIDs), etc.

Thus, in persons with ulcerative diseases, who take ASA, the frequency of the development of gastrointestinal bleeding (GIB) increases by 3 times. The use of NSAIDs in combination with ASA raises the probability of the emergence of GIB approximately by 4 times as compared to ASA monotherapy. Simultaneously, the indication of ASA during 26 weeks together with PPIs, for example, esomeprazol with the dose of 20 mg per 24 hours, decreased the risk of the stomach and duodenum ulcers development from 5,4 to 1,6% [5,6].

In 2002, in the manual issued by the American College of Cardiology (ACC) and American Heart Association (AHA), it was noted that in case of the impossibility of ASA application as a consequence of its negative impact on the GIT, one should use clopidogrel.

The recommendation like this was shaped on the basis of the CARPIE research, in which they compared the effects of ASA(325 mg/24 h) and clopidogrel (75 mg /24 h) in patients with the risk of ischemic events .In both groups, the frequency of GIB was low: in the ASA – 0,72%, in the clopidogrel – 0,52%, However, it was concluded that the latter had some advantages [7,8,9].

Nevertheless, the subsequent researches with the more

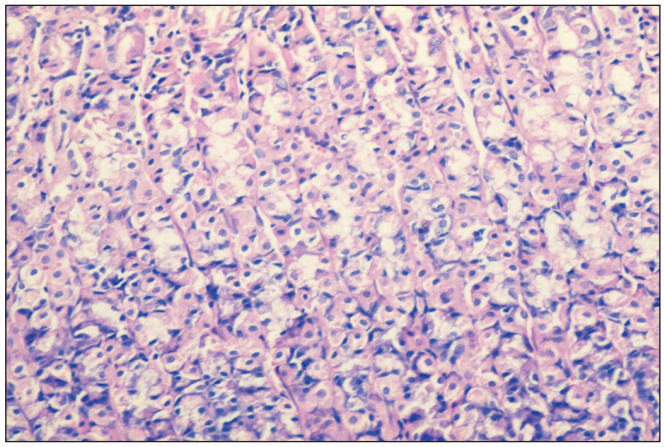

Fig. 1. The mucosa of the fundic part of the rat stomach (control group, the 14th day). The histological structure of the GM is preserved with the distinct differentiated location of the fundic glands' cells. Staining with hematoxyllin and eosin x 100.

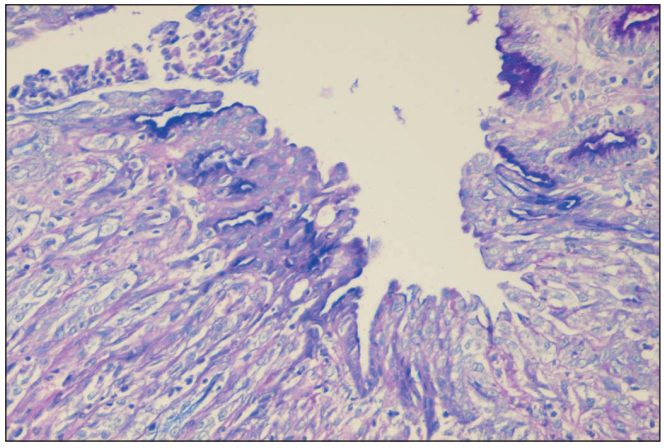

Fig. 3. Superficial erosion of antral part GM rat. Desquamative epithelium, the absence of neutral glycoproteins, an unsignificant number of acid sialomucins (blue color). ASA, 14 day. Combined PAS-reaction and alcian blue x 400.

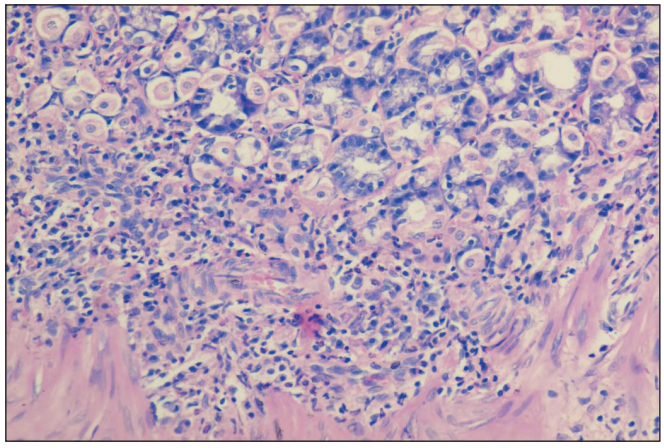

Fig. 5. The dissociation of parietal and chief exocrinocytes in the basal parts of GM. Staining with hematoxyllin and eosin x 400.

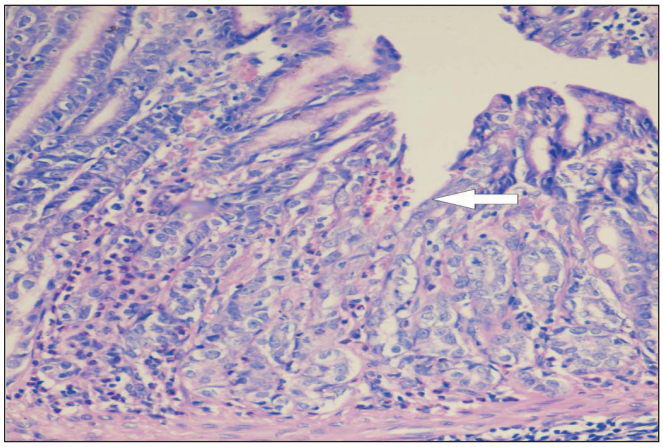

Fig. 2. Superficial erosion of the rat's GM antral part (arrow). Minute haemorrhage, single leukocytes. ASA, 14 day. Staining with hematoxyllin and eosin x 200.

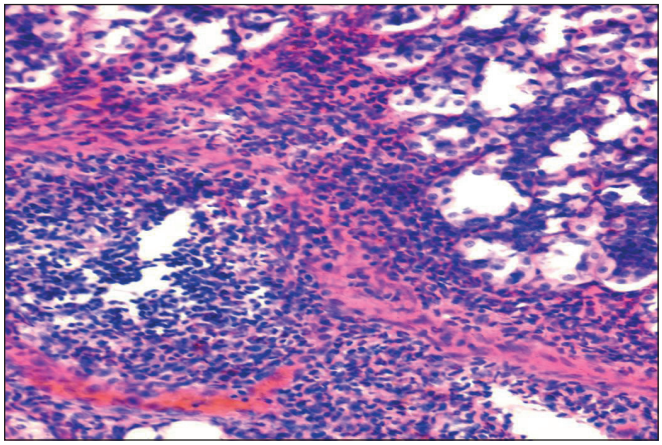

Fig. 4. The focal lympho- and plasmocytic infiltration of the GM muscular lamina. ASA, 14 days. Staining with hematoxyllin and eosin x 400.

detailed design proved the ulcerogenic effect of antiaggregants. In the case-control study, which included 2777 patients with expressed GIB and 5532 patients of the control group, it was stated that clopidogrel and ticlopidine have a comparable relative risk equal to 2,8 in relation to ASA and anticoagulants [10].

Antiaggregants are as dangerous as low doses of ASA as for the risk of GIB development. It is better to prescribe low doses of ASA with PPIs rather than clopidogrel to patients with high GIB risk [11].

Low doses of ASA used as an antiaggregant remedy in cardiovascular diseases (CVD) can also cause the development of serious gastroduodenal complications. In 30-40% of the patients, who took ASA in the minimum dose of 75 mg/24 h, gastric erosions emerged. After the literature in question data, the use of ASA in such a dose doubles the risk of GIB [12, 13].

The leading role in the treatment of NSAIDs gastropathies is played by antisecretory preparations which reduce the damaging effect of the hydrochloric acid and pepsin which is the main factor of aggression in the pathogenesis of the ulcerative and erosive damage to the mucosa of the upper parts of the GIT.

The best results on tolerance among PPIs are demonstrated by pantoprazole: in its application, insignificant side effects are recorded just in 1,1% patients [14, 15].

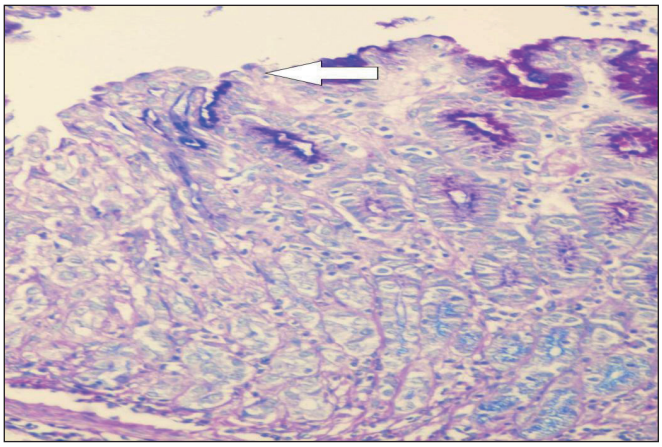

Fig. 6. Superficial erosion of the antral part GM. The basement membrane is absent in the area of damage (marked by an arrow) and preserved in the foci of neutral mucins accumulation (crimson colour). ASA, 14 days. Combined PAS-reaction–alcian blue x 400.

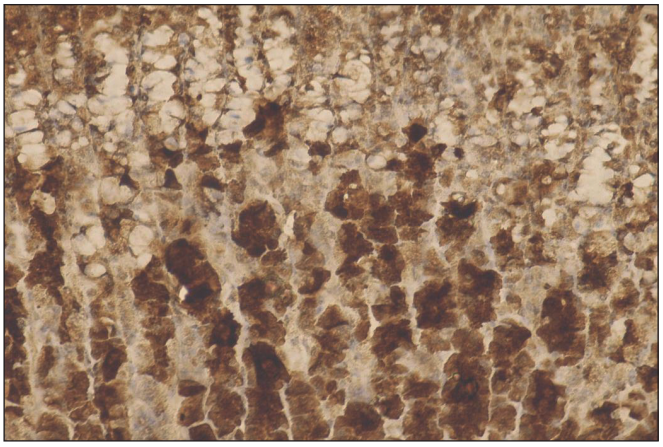

Fig. 7. A strong expressionofcaspase-3 (CPP32), mainly in the basal parts of GM. ASA + clopidogrel, 14day. Immunohistochemical reaction CPP32 x 200.

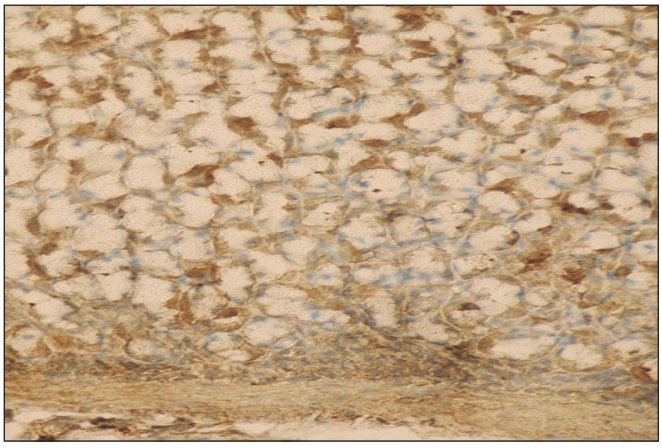

Fig. 8. The moderate expression of CPP32 in perinuclear parts of fundic glands exocrinocytes. Clopidogrel + pantoprazole, 14day. Immunohistochemical reaction CPP32 x 200.

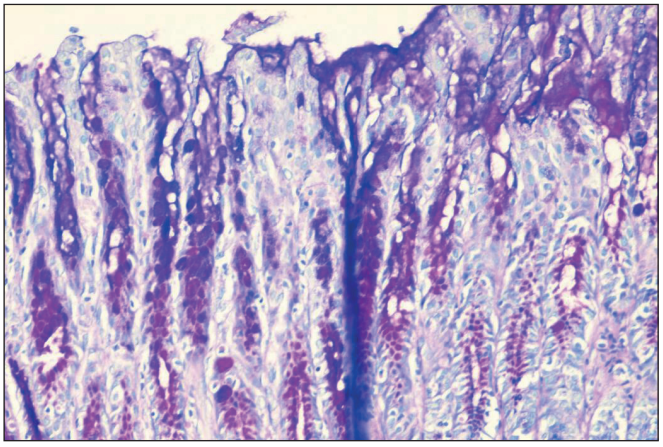

Fig. 9. The appearance ofacidsialomucinsin the superficial epitheliocytes of rat'sGM. ASA +clopidogrel+pantoprazole, 14day. A combined PAS-reaction with alcian blue x 200.

The modern diagnostics of NSAIDs gastropathies represents an unsolved finally problem connected to the peculiarities their clinic manifestations. For 30-90%, their asymptomatic course is characteristic, while for 46-58% – the absence of typical and expressed clinical picture and in 25-42% patients symptoms of the basic diseases prevail. As a consequence of this, NSAIDs gastropathies often remain undiagnosed till they appear as bleeding not rarely dangerous for life.

That is, the problem of NSAIDs' application in the form of monotherapy or in combination with other groups of antiaggregants in patients with cardio-vascular diseases (CVD) has not been solved eventually by now.

The correlation of aggressive factors of gastric contents and protective systems of gastric mucosa (GM) in the application of ASA and clopidogrel are still little studied.

The morphological criteria of the gastropathies induced by ASA and clopidogrel need further research. Hence, the specification of NSAIDs-induced gastropathies' mechanisms and the search of effective ways of their prophylaxes and treatment are actual.

As of today, reliable trustworthy morphological criteria of NSAIDs gastropathies which enable to differentiate them from gastritis are absent.

One of the basic method of NSAIDs-gastropathies diagnostics is morphological one but, gastropathies have no specific signs. That is why the aim of the research was to find out the pathomorphological peculiarities of the gastric mucosa at early stage of prescription of both ASA and combined therapy of ASA and clopidogrel as well as to study the impact of pantoprazole on the gastric mucosa to optimize the prophylaxis and treatment of gastropathies induced by ASA and clopidogrel.

THE AIM

The aim was to characterize the morphological peculiarities of the gastric mucosa at early stage of prescription of acetylsalicylic acid (ASA) and clopidogrel as well as to study the impact of pantoprazole on the gastric mucosa to optimize the prophylaxis and treatment of gastropathies induced by ASA and clopidogrel.

Table I. Apoptosis index of gastric epitheliocytes

Groups	Apoptosis index (%)
Control	2,9±0,32
ASA	7,18±0,48**
Clopidogrel	4,6±0,61*
ASA+clopidogrel	9,3±0,37**
ASA+pantoprazole	4,1±0,56
Clopidogrel + pantoprazole	3,5±0,42
ASA+ clopidogrel + pantoprazole	3,4±0,31

* - p<0,05 in comparison to control group;
** - p<0,001 in comparison to control group.

MATERIALS AND METHODS

The research was carried out at the Histology, Forensic Medicine and Law Department of Vinnytsya National Pirogov Memorial Medical University.

The experiments were performed on 77 non-linear white male rats with the average weight of 150-180 g. Depending on the aim of research, the animals were divided into 7 groups. 5 animals made the control group, while the rest 72 once were divided into 6 groups (12 animals in each).

1. Group ASA took acetylsalicylic acid in the dose of 6,8 mg/kg of the body weight (translation for an adult – 75 mg).
2. Group got clopidogrel 8 mg/kg of the body weight (translation for an adult – 75 mg).
3. Group ASA + clopidogrel got clopidogrel 6,8 mg/kg of the body weight (translation for an adult – 75 mg).
4. Group ASA + pantoprazole received acid 6,8 mg/kg of the body weight + pantoprazole 3,6 mg/kg (translation for an adult – 40 mg).
5. Group clopidogrel + pantoprazole was given clopidogrel 6,8 mg/kg of the body weight and pantoprazole 3,6 mg/kg.
6. Group ASA + clopidogrel + pantoprazole got acetylsalicylic acid 6,8 mg/kg of the body weight, clopidogrel 6,8 mg/kg of the body weight and pantoprazole 3,6 mg/kg.

The doses and ways of introduction of the mentioned substances were selected according to their clinical application and translated in relation to rats after the recommendations given by [16].

The duration of the experimental research was 14 days. During the research all the animals were given a standard ration of vivarium; the eating pattern and water consumption were ad libitum. The drugs were introduced once in 24 hours intragastrally with the help of a metal probe per 1% of starch suspension.

The experimient was done according to the rules of humane treatment of experimental animals confirmed by the Bioethics Committee of Vinnytsya National Pirogov Memorial Medical University.

The animals were excluded from the experiment through the overdosing of thiopental anaesthesia. The material obtained (stomachs) were fixed in 10% neutral formalin and after commonly accepted processing paraffin blocks were made, of which, in turn, sections of 5-7 mkm thickness were produced of the fundal and antral parts of the stomach by 2 pieces respectively.

To define pathomorphological changes, general histological techniques were applied (staining with hematoxylin and eosin), while to identify acid and neutral glycoproteins histochemical techniques were used (staining with alcian blue in pH 1,0 and 2,5 in combination with PAS-reaction) [1].

For the quantitative assessment of apoptotic cells' contents, the index of apoptosis (IA) was used, which was calculated in percents after the formula IA = the number of apoptotic cells/general number of all cells x 100 [17].

The immunohistochemical research was carried out on paraffin sections with the use of the streptavidin-biotin method («DAKO», Denmark, LSAB2 Systems, HRP).

A damasking of antigen was performed in the citrate buffer with pH 6,0. Mouse monoclonal antibodies as primary ones were used. The cells nuclei were additionally stained with Mayer's hematoxylin during 15-60 sec.

The expression of caspase-3 was evaluated with the help of mouse monoclonal antibodies CPP32 (clone JHM62, «Novocastra», Great Britain). To identify the expression of CPP32, the semi-quantitative scale of the evaluation of staining intensity was employed: 0 (absent) – the absence of positive reaction in cells, 1 (weak) – up to 30% of cells which reacted positively, 2 (moderate) – 31–60%, 3 (strong) – 60% and more of stained cells [18].

RESULTS

In visual microscopy of GM in the control group, gastric pits were placed closely to each other, were not profound, straight, covered with cylindrical epithelium with basally located oval nuclei; in this, 2-3 fundic glands'necks entered each gastric pit.

The fundic glands looked like compact groups divided by thin layers of connective tissue. Their lumens were narrow. In the structure of the fundic glands, 3 main parts of cellular elements were defined clearly enough: chief, parietal exocrinocytes and mucous neck cells (Fig. 1).

The groups of experimental animals, which received ASA + pantoprazole and clopidogrel + pantoprazole, were characterized by the preservation of the basement membrane, which was clearly eminent under the superficial epithelium around the glands and was attached by blood capillaries.

The connective tissue of GM was denudated within a short extension in both the ASA and clopidogrel groups as well; microerosions formed mainly in the antral part (in 94% of experimental animals).

The bottom of microerosions contained swollen connective tissue. The subepithelial lamina propria was marked by minute diapedetic hemorrhages, while infiltration by single lymphocytes and plasma cells around the area of swelling was noticeable (Fig. 2).

The denudated connective tissue of the lamina propria of GM was just covered with a thin layer of mucosa, which was locally absent. It was well observed while using PAS-reaction in combination with alcian blue (Fig. 3).

Interestingly, in 42% experimental animals, both of the ASA and clopidogrel groups, we observed the focal lympho and-plasmocytic infiltration with admixtures of segmentonuclear leukocytes in the basal parts of GM on the border with the muscular lamina (Fig. 4) and the dissociation of parietal and chief exocrinocytes (Fig. 5).

Damage to the basement membrane of superficial epitheliocytes, which was poorly enough diagnosed while staining with hematoxyllin and eosin and was clearly revealed in using of PAS-reaction, became one of the characteristic features for the ASA and clopidogrel groups separately as well as for the ASA+ clopidogrel group (Fig. 6).

However, in the group of ASA + clopidogrel damage to basement membrane was revealed in 42% experimental animals as opposed to 25% of the ASA group and 10% of the clopidogrel group and was not observed in the groups which obtained ASA + pantoprazole, clopidogrel + pantoprazole and ASA + clopidogrel + pantoprazole.

It is known that the basement membrane of the epithelial cover is formed of proteins synthesised by epitheliocytes. In the situation described above, the focal absence of the GM basement membrane epithelium can be connected to immaturity of epitheliocytes as a consequence of their accelerated death and regeneration which was confirmed by us in using caspase-3 (CPP32).

In particular, in the ASA group and the ASA + clopidogrel the number of positively stain cells made over 70% (fig. 7), while in the control group they made up to 10%. The difference between the groups was reliable ($p<0,05$).

While in the ASA + pantoprazole, clopidogrel + pantoprazole, ASA + clopidogrel + pantoprazole groups positively stained cells made 18%, 12% and 15% (fig. 8), respectively, the differences within the groups being unreliable($p>0,05$).

In the ASA groups uperficial microerosions were observed in 25% of cases while in the ASA +clopidogrel group – in 42 %. However, in the clopidogrel group they were revealed just in 10% of the experimental animals.

After localization, microerosions, which dominated in the antral part (94%), were observed. In the contol groups, ASA + pantoprazole, clopidogrel + pantoprazole, ASA + clopidogrel + pantoprazole erosions were not found.

Thus, in the ASA, clopidogrel and ASA + clopidogrel groups, homogenous changes in rats' GM and desquamation of superficial epitheliocytes were found. In this, the desquamation of superficial epitheliocytes with the breaking of the basement membrane integrity and formation of microerosions were revealed, degenerative disturbances being predominant. It was well revealed in using PAS-reaction with alcian blue.In the control group, the superficial epithelium had a highly prismatic form common to it and contained a large amount of secret.

At the same time, in the group of experimental animals, who received ASA + clopidogrel + pantoprazole with a higher frequency than that in the control one, different intensity of chief and parietal exocrinocytes staining was revealed. This probably depended on the different functional state of these glandular components. The number of acid sialomucins grew in superficial epitheliocytes as well (Fig. 9).

In the fundic part of the animals the superficial part of the GM preserved its usual structure but after the histochemical investigation in the most differentiated part and in the area of localization of the terminal parts somepeculiarities were determined.

Thus, in the group of clopidogrel the PAS-positive secrete granules in the chief cells were revealed. At the same time the cytoplasm of some chief cells lost its ability to stain and becomes cleared especially around the nucleus and between nucleus andthe basement membrane. The height of these cells decreases and nuclei become wrinkled with pyknosis.

The substantial difference in the distribution of mucus granules in the cytoplasm of superficial epitheliocytes in the ASA + pantoprazole, clopidogrel + pantoprazole, ASA + clopidogrel + pantoprazole was not not as well as in the control group.

Mucous neck cells of glands have less sizes than superficial epithelium and in the basal part of the latter the round nuclei were detected. The intensity of PAS-reaction with a homogeneous distribution of mucous secretion in these cells cytoplasm were smaller than in the superficial epithelium.

The structure of the other parts of the oxyntic and pyloric glands in the control group of animals and ASA + pantoprazole i clopidogrel + pantoprazole groups don't differ by any peculiarities when we use the conventional methods on the light level.

However, the essential decrease of epithelium secretory activity in the groups of ASA, clopidogrel and ASA + clopidogrel was revealed. In some areas adjacent to microerosions we detected even full lack of secretion. In that areas only remains of mucous substances (mainly acid glycoproteins) were observed using histhochemical method.

DISCUSSION

Thus, the pathomorphological changes in an erosive lesion of GM characterized by the mucins secretion disturbance of surface and foveolar epithelium, infiltration of lamina propria of GM by mononuclear cells and neutrophilic leucocytes and absence or small zone of fibrinoid necrosis. The disturbance of mucus secretion and glands destruction was associated with inflammatory infiltration in 19% of

ASA + clopidogrel group, 15% of animals of ASA and 2% of clopidogrel group.

The same phenomena which almost related to the damage of glandular epithelium were found in the stomach antral part that was concerned more inflammation activity in particular this part of the stomach. In the clopidogrel group the glands surrounding erosion of GM for the most part preserved their normal structure and histochemical properties.

In the group of experimental animals that received the ASA the microerosions were as superficial epithelial defects with serous stoma swelling and cellular infiltrate (mainly neutrophilic leucocytes).

The field of fibrinoid necrosis was represented by unstructured weak eosinophilic PAS-positive masses with bord er parallel to surface of GM however they were observed only in 17% cases of ASA group and in 4% of the clopidogrel one.

From the direction of vascular bed the swelling of vascular walls and endotheliocytes with nuclei vacuolization, detachment of them into vessels lumen, increase permeability with perivascular edema, extravasates and plasmorrhagia was observed.

Thus, the histochemical data obtained by us suggest that administration of ASA, clopidogrel and combination thereof without prescription of concomitant Pantaprozole significantly decreased the synthesis of neutral glycoproteins and increased the synthesis of acid glycoproteins.

Blockage of enzyme systems of mitochondria epitheliocytes, which causes a disturbance of the processes of oxidative phosphorylation and leads to development of a cascade of non-fibrotic processes in cells, may be one of the important points in the pathogenesis of ASA and clopidogrel contact action. Inhibition of prostaglandin (PG) synthesis due to inhibition of cyclooxygenase (COX1) leads to a decreased secretion of the mucous gel, reduced secretion of bicarbonates, deterioration of GM blood supply, resulting in a disturbance of protective and reparative properties of its cells, which leads to emergence of erosions and ulcers. Probably, PG is a trigger mechanism for GM trophicity disorder, which leads to indurative changes and cellular destruction associated with onset of erosion [3, 19, 20, 21].

In addition, the medicines of this group have the ability to directly penetrate into the cells of the mucous membrane in the acidic gastric medium, breaking the mucosal-bicarbonate barrier and causing the inverse diffusion of hydrogen ions, thus damaging the cells of the envelope epithelium. Products of free-radical oxidation of lipids involved in damaging the mucous membrane of the stomach and destruction of mucopolysaccharides are formed as a result of ASA toxic effects. It has been proved that some NSAIDs can directly inhibit mucin synthesis and bicarbonate secretion, as chemical bond of these substances with gastric mucosal phospholipids reduces its hydrophobicity. NSAIDs influence the proliferation of epithelial cells, reducing the protective properties of the epithelium and the ability for adequate reparation [22, 23, 24, 25, 26].

Pantoprazole supports the prevention of GM necrotic lesion by enhancing the protective properties of mucus. This phenomenon is caused by an increase of production of protective PGs, higher accessibility of sulfhydryl radicals, and reduced duration of antioxidant stress at GM level [15, 27, 28, 29].

During the apoptotic changes analysis of GM in different groups of animals the certain patterns were established. In particular in the control group the index of apoptosis to average 2,9±0,32% while in clopidogrel group it was 4,6±0,61%, ASA – 7,18±0,48% and as much as possible the index of apoptosis was observed in ASA + clopidogrel 9,3±0,37% group (Table 1).

It should noted that the reliable differences of the apoptosis index in the control group when compared with clopidogrel ($p<0,05$), ASA ($p<0,001$) and ASA + clopidogrel groups ($p<0,001$).

The index of apoptosis was significantly lower in comparison to ASA and ASA + clopidogrel groups that pointing out less negative influence of clopidogrel on the GM in contrast to ASA and combination ASA + clopidogrel.

In clopidogrel + pantoprazole, ASA + pantoprazole and ASA + clopidogrel + pantoprazole groups the index of apoptosis amounts to 3,5±0,42%, 4,1±0,56% and 3,4±0,31%correspondingly. We don't establish the reliable differences these groups when compared to control group ($p>0,05$). The differences were absent also when we make a comparison of the apoptosis index between clopidogrel + pantoprazole, ASA + pantoprazole abd ASA + clopidogrel + pantoprazole

By this line of reasoning, we conclude that pantoprazole has a positive effect on the GM epithelium during the clopidogrel, ASA antiplatelet pharmacotherapy performance as well as using of combination ASA with clopidogrel.

Thus, the problem remains relevant for safe using of ASA and clopidogrel and will demand the new searches and investigations in the future. The scientific searches directed to study of the blood circulation state, mechanisms of angionesis, balance of proliferation and apoptosis of gastric epitheliocytes, epidermal and transforming growth factors and deep understanding of molecular mechanisms enable to create the new derivatives of NSAIDs and antiaggregants targeted on certain elements of cells signal system.

CONCLUSIONS

1. According to our study findings, administration of ASA in combination with clopidogrel results in 2,5 times higher risk of GM erosive lesions.
2. One of the most significant morphological manifestations of gastropathy in ASA and clopidogrel regimen is the development of microerosions, which are poorly diagnosed by macroscopic examination.
3. The use of PAS-reaction makes possible to identify damage to the basal membrane of superficial epitheliocytes, which may be a top-priority morphological criterion of gastropathy induced by ASA or clopidogrel in the absence of an inflammatory reaction.

4. Administration of pantoprazole in combination with ASA and clopidogrel contributes to preventing GM necrotic lesions by amplification of protective properties of mucus and stabilization of apoptotic activity of gastric epithelial cells.

Further study and regard to risk factors of development of gastropathy associated with administration of ASA and clopidogrel will provide an opportunity to significantly reduce the incidence of gastroduodenal complications and improve the expected effects of the above medicines.

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The Authors declare no conflict of interest.

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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

ASSESSMENT OF CORRELATION BETWEEN MIRNAS-21-3P AND -210-3P EXPRESSION IN MATERNAL AND UMBILICAL CORD PLASMA AND FETAL WEIGHT AT BIRTH

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ABSTRACT

The aim: To determine the degree of correlation of mass of the fetus and the level of mir-21, mir210 in maternal blood and umbilical cord blood of the fetus in uncomplicated gestation.

Materials and methods: 60 pregnant women with a single baby pregnancy in the third trimester (37-40 weeks) were examined. They all were given a general clinical, obstetric and the level of miRNA21-3p and miRNA210-3p were determined in the whole blood of pregnant women (before labor) and in fetal blood obtained from the umbilical artery at birth. The level of miRNAs was determined by the TaqMan method.

Results: After examining maternal and fetal plasma samples, we were able to determine 49 samples of hsa-miR210-3p and hsa-miR21-3p from maternal plasma, 44 samples of hsa-miR210-3p and 37 samples of hsa-miR21-3p from the cord blood, which is a satisfactory result of more than 50%. Subsequently, between the results obtained and the birth weight of the fetus Pearson's correlation coefficient was studied. According to the results obtained, we found no correlation between fetal mass and hsa-miR210-3p level in maternal plasma (r-0,068674), low positive correlation of fetal mass with hsa-miR21-3p level in maternal plasma (r-0,212181), an average positive correlation with the level of hsa-miR21-3p in umbilical cord blood (r- 0.363374) and a high positive correlation with hsa-miR210-3p in umbilical cord blood (r-0.528616).

Conclusions: Determination of the level of hypoxic miRNAs, in particular hsa-miR210-3p in the umbilical cord blood of the newborn may be a marker of the functional status of the placenta, which programs the normal development of the fetus.

KEY WORDS: miRNA, Hypoxia, pregnancy, fetal programming, newborn

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INTRODUCTION

In the mid-80s (1980-ies), the results of a research by D. Barker were published that laid the basis of prenatal programming theory. The essence of the problem is that the weight of a child at birth can be a marker of further development of its numerous diseases in the adulthood, namely non-insulin-dependent diabetes mellitus, hypertension, cancer, obesity, as well other pathological states that might even determine the life expectancy of an individual [1].

It was considered that the potential for prenatal development, in particular fetal mass, was formed due to one's genetics. However, according to the literature data as of now, approximately 20% of its variability is due to maternal factors, and much less contribution is made by genetic variants of a fetal genome [2]. Pathology in course of a pregnancy, stress, influence of mother's nutrition and other environmental factors become those triggers that alter gene expression and determine its phenotype. That is, gene reprogramming without changing the structure of DNA determines the structural and functional features of the fetus development and the prognosis of its further state of health.

The main mechanism that mediates changes in the trajectory of growth and formation of the fetus is hypoxia. It has a direct physiological effect on the development of the fetus, but it can also indirectly modulate it through interference with a placenta, as well as via angiogenesis and hematopoesis processes. It is hypoxia that determines which way it would go: normal or pathological. Low oxygenation activates reactions that are regulated by HIF1α (hypoxia-induced factor 1α), at least many of them do. It is involved in cellular control of the utilization and delivery of O_2, its inhibition of growth and development through alteration of gene expression. Also, it promotes anaerobic metabolism pathways [3].

In this regard, the study of non-invasive molecular markers of hypoxia is rising up as a promising area of a fetal medicine. These studies should promote better understanding the pathophysiology of intrauterine hypoxia and fetal programming, as well as to identify its new markers, more accurately predicting the short-term and long-term complications, and monitoring and choosing rational tactics for managing this pregnancy.

New markers of fetal programming include the class of microRNAs that are small non-coding RNA molecules of 18–25 nucleotides (total length's average of 22), which are involved in transcriptional and posttranscriptional regulation of gene expression. In human organism approximately 2000 of them have been determined. Some of them can be allocated from the tissues only, and that fact

complicates studying them. The rest are excreted into the bloodstream in the form of membrane microvesicles or necrotic nanoparticles and are available for determination.

MicroRNAs regulate virtually all biological and metabolic processes in the body, including the development of normal and pathological pregnancy. Determining them from the bloodstream of the mother, as well as from placenta, amniotic fluid and umbilical cord blood allows the researcher to use them as markers of pregnancy miscarriage, preeclampsia, gestational diabetes, macrosomia, delayed fetal development, etcetera. Yet the results are quite controversial due to relatively high costs of microRNA determination, different methodological approaches to their determination, small number of studies undertaken and, thus, the small number of the results obtained.

Among the first chronologically determined and most studied were hypoxic micro-RNAs, specifically mir-21 and mir-210. They circulate, lack tissue-specificity and allow a comprehensive assessment of the body's response to low O_2 concentrations during pregnancy.

When summarizing the available literature data, it is often noted that the presence of miR-21 and mir-210 miRNAs in maternal blood correlates with the degree of fetal hypoxia in utero, and thus they are considered as its markers [4]. However, we did not find neither any works on parallel study for both mother and fetus, nor the study on the presence or absence of relation with the mass of the fetus.

THE AIM

Therefore**,** the issue of our study was to determine the degree of correlation of mass of the fetus and the level of mir-21, mir210 in maternal blood and umbilical cord blood of the fetus in uncomplicated gestation.

MATERIALS AND METHODS

60 pregnant women with a single baby pregnancy in the third trimester (37-40 weeks) were examined.

Inclusion criteria: full-term pregnancy (37-42 weeks), single fetal pregnancy, absence of extragenital pathology, inflammation or preeclampsia, nonsmoking, satisfactory condition of the fetus, agreement of patient.

Exclusion criteria: pregnancy period <37 weeks or> 42 weeks, multi fetal pregnancy, presence of extragenital pathology, inflammation or preeclampsia, smoking, fetal distress, disagreement of patient.

They all were given a general clinical, obstetric and the level of miRNA21-3p and miRNA210-3p were determined in the whole blood of pregnant women (before labor) and in fetal blood obtained from the umbilical artery at birth. The material was a heparinized blood.

The level of hsa-miR-21 and hsa-miR-210 was determined by the TaqMan method.The total RNA was isolated from the blood using the mirVana PARIS method (Ambion, USA) in accordance with the manufacture's protocols. The concentration of RNA was measured with a spectrophotometer NanoDrop ND1000 (NanoDrop Technologies, USA). MiRNAs were identified by a reverse transcription and PCR in real time. The reverse transcription was conducted with High Capacity cDNA Reverse Transcription Kit (Applied Byosystems, USA), with specific primers for every miRNA and 10 ng of total RNA. The quantitative PCR in real time was conducted using TaqMan MicroRNA Assays (Applied Biosystems, USA): U6 snRNA (as endogenous control), assay ID 002438 (hsa-miR21-3p), assay ID 000512 (hsa-miR210-3p). The temperature conditions were as follows: initial denaturation 95°C – 10 min; 45 cycles 95°C – 15 s and 60°C – 60 s. The level of miRNA was determined using the formula ($2^{\Delta Ct}$), normalized according U6 snRNA and is expressed in relative units. Amplification was carried out with 7500 Fast Real-time PCR (Applied Byosystems, USA).The data received was analyzed using 7500 Fast Real time PCR software.

Statistical Analysis. All values are presented as arithmetic mean ± standard deviation (S.D.) Data in all groups were analysis of variance was performed using one-way ANOVA. The software used was Excel 2007 and SPSS Statistics 17.0. Data obtained were analyzed using Microsoft Office 2007 *и* Statistica 6 (StatSoft Inc., USA) programs.

The experimental results received were compared by determining the correlation coefficient between levels of microRNA in womens blood, umbilical blood and newborn weight.

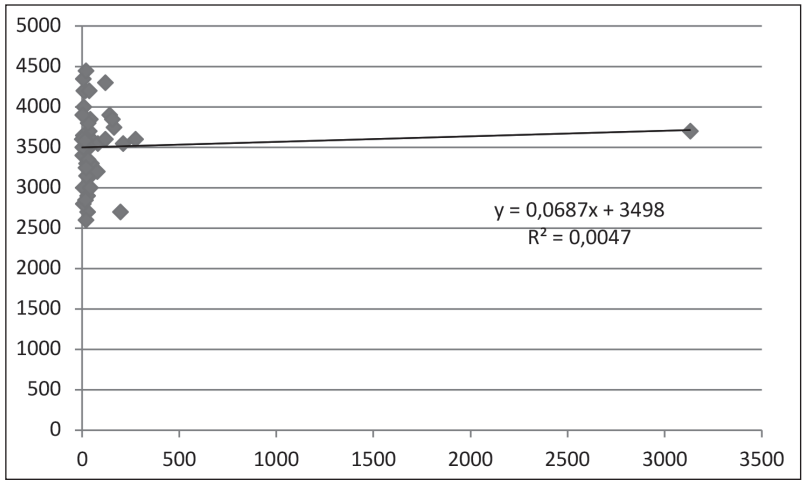

Fig. 1. Correlation between miRNAs-210-3p expression in maternal plasma and fetal weight at birth.

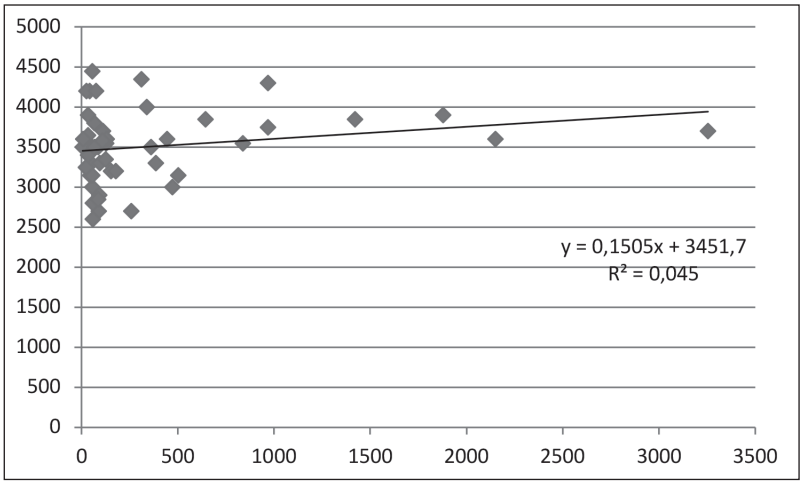

Fig. 2. Correlation between miRNAs-21-3p expression in maternal plasma and fetal weight at birth.

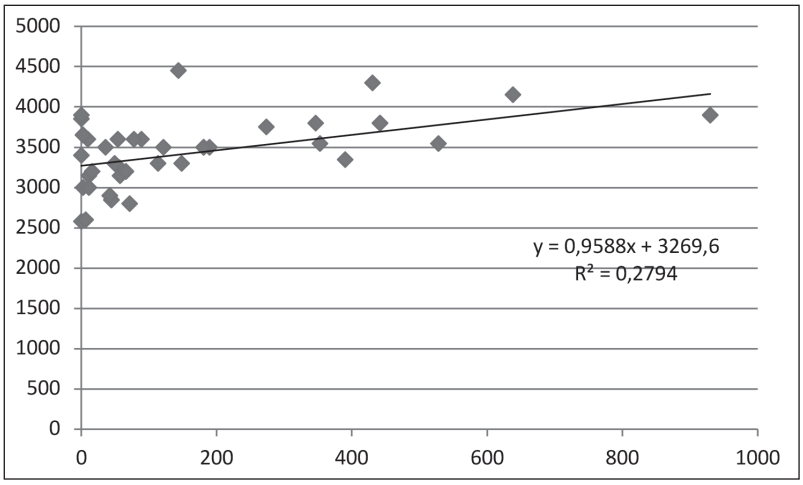

Fig. 3. Correlation between miRNAs-210-3p expression in umbilical cord plasma and fetal weight at birth

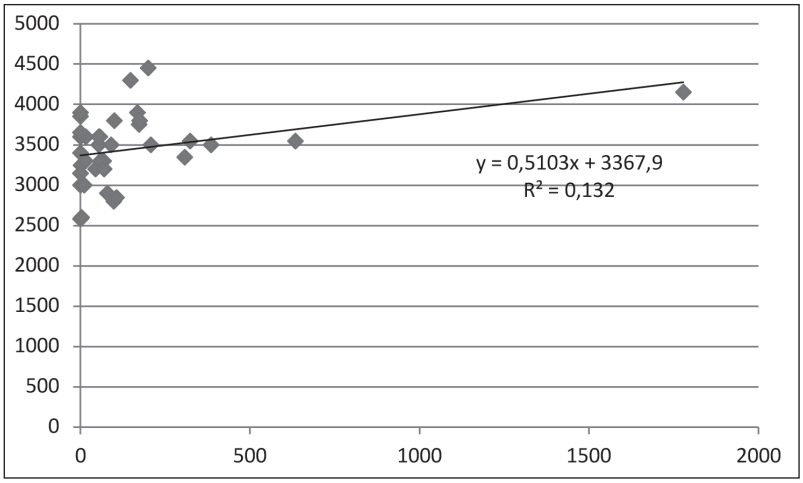

Fig. 4. Correlation between miRNAs-21-3p expression in umbilical cord plasma and fetal weight at birth.

RESULTS

The study was conducted in 60 pregnant women. The mean age of pregnant women was 28.32 years ± 4.6 years. 55 women (91.7%) of these were carrying their first child, 5 women (8.3%) had already given birth before. Vaginal delivery – 58 women (96.7%), and 2 (3.3%) underwent caesarean section because of the clinical mismatch between the size of the fetus and pelvis of the mother.

After examining maternal and fetal plasma samples, we were able to determine 49 samples of hsa-miR210-3p and hsa-miR21-3p from maternal plasma, 44 samples of hsa-miR210-3p and 37 samples of hsa-miR21-3p from the cord blood, which is a satisfactory result of more than 50%. Subsequently, between the results obtained and the birth weight of the fetus Pearson's correlation coefficient was studied. The results obtained are presented in Figgs 1-4.

According to the results obtained, we found no correlation between fetal weight and hsa-miR210-3p level in maternal plasma (r-0,068674), low positive correlation of fetal weight with hsa-miR21-3p level in maternal plasma

(r-0,212181), an average positive correlation with the level of hsa-miR21-3p in umbilical cord blood (r- 0.363374) and a high positive correlation with hsa-miR210-3p in umbilical cord blood (r-0.528616).

DISCUSSION

The relevance and prospects of epigenetic studies, as well as the widespread use of PCR (polymerase chain reaction) in clinical practice and the focus of research on the simplification of microRNA determination make it possible to conduct this study. But the questions regarding its appropriateness arise. The presence of medium and high correlation between hypoxic miRNAs and its mass in the umbilical cord blood of the newborn only, and the negative result of the study of maternal blood have no prognostic value for us with respect to fetal weight. But on the other hand, it allows answering the certain pathogenetic questions.

The data obtained by us contradicts the literature data. In particular, Whitehead C. et.al. determined the miRNAs of placental origin in maternal blood. According to their results, the level of hypoxic miRNAs in maternal blood flow, in particular miR-210, correlates with the acidic status of fetuses with intrauterine growth restriction (IUGR) in acute fetal hypoxia in the second period of labor, and chronic hypoxia with premature delivery of fetuses with IUGR [5]. However, the investigated miRNAs do not have rigid tissue specificity, and according to the study, it is not possible to clearly identify whether they belong to a mother or a fetus. Therefore, their level in the circulation of a mother is an aggregated value for the maternal organism and the feto-placental complex, largely reflecting the condition of the pregnant woman and the pathology present.

The umbilical cord artery reflects the state of placental microcirculation and its functioning more precisely. Thus, the present correlation of angio-microRNA (hsa-miR210-3p hsa-miR21-3p) in the umbilical cord blood with the newborn's mass may indicate the degree of placental dysfunction, which is key factor in terms of pregnancy outcome and subsequent prognosis for the baby.

On the basis of the numerous studies, microRNAs that are also called angio-microRNAs have been found to play an important role in placental angiogenesis. For instance, miR-21, miR-20a and miR-210 have been shown to induce angiogenesis, whereas miR-16, miR-34a and miR-222 inhibit angiogenesis. In the placenta, miR-20a and miR-34a were identified in helix remodeling, miR-16 in the vascular endothelial growth factor signaling pathway, and miR-210 in trophoblast migration and invasion. In the case of imbalance between pro- and anti-angiogenic factors, there may be an increase or decrease in the lumen of the blood vessels, which can lead to abnormal development of the placenta. In addition, oxidative stress is involved in placental vessel dysfunction [6]. The aforementioned miRNAs are involved in fundamental biological processes such as inflammation (miR-21), regulation of the cell cycle (miR-21, miR-210), apoptosis (miR-21), oxidative stress (miR-210) and cellular senescence (miR- 21, miR-210) [7].

Almost all available studies in this field are being conducted with placental tissue and in low-weight fetuses (intrauterine growth restriction). But the results are also quite controversial. For example, Cindrova-Davies T. et. al. determine increased expression of miR-21 in cases of preeclampsia and IUGR [8]. At the same time, the decreased expression of placental *miR-21* in combination with *miR-16* was determined by Maccani, M. A et.al. in children with extremely low weight at birth. Moreover, the simultaneous decrease in their level has a greater prognostic result, than a simple low expression of each of them individually do. That suggests an additive effect [9].

Zhang, J. et al found an increase in the expression of miR – 21 in macrosomal placenta in comparison with control samples [10]. Jiang, H. et. al. suggest that its activation in placenta can cause macrosomia via stimulating cellular differentiation and proliferation [11]. miR-21 regulates adipocyte differentiation and proliferation, and its expression correlates positively with body mass index [12]. That is, the miR-21 study makes it possible to evaluate the complex impact of many factors of mainly placental origin on fetal mass.

A placenta determines the growth and development of the fetus, and the development of placenta is controlled by the level of oxygen tension. Hypoxia-induced factor 1α (HIF1α) plays an important role in the control of placental oxygenation [13]. HIF directly targets vascular endothelial growth factor (VEGF), namely a major regulator of angiogenesis. MiR-210 was one of the first hypoxic miRNAs to be detected as a direct transcriptional target of HIF. Under normoxic conditions miR-210 is expressed at low levels, especially in certain cell types, such as endothelial cells. Under the influence of hypoxia, the transcription of miR-210 is dynamically activated in all known mammalian cell types, primarily through HIF-1α interaction. MiR-210 also reduces the need for cellular energy, causing hypoxic cell growth to be stopped by influencing the transcription factor E2F (E2F3), a cell cycle regulator not directly regulated by HIF transcription [14]. HIF1α not only induces the expression of miRNA -210, but in turn, it has been shown that this miRNA inhibits HIF1α. Hypoxia determines the expression of HIF1α and some of its target genes / miRNA -210 and their association with fetal growth parameters. During normal healthy pregnancy the expression of miR-210 remains at a fairly constant level [15]. Thus, the presence of a high level of correlation of hsa-miR210-3p in the umbilical cord blood of the newborn may be a marker of the functional status of the placenta, which programs the normal development of the fetus.

CONCLUSIONS

Hsa-miR210-3p potentially holds some interest for clinical practice, as possible markers of hypoxia both past and current, as well as its severity and effects.

Determination of the level of hypoxic miRNAs, in particular hsa-miR210-3p in the umbilical cord blood of the newborn may be a marker of the functional status of the placenta, which programs the normal development

of the fetus. But this study provides only partial answers to questions regarding the importance of hypoxia in programming of the fetal development. For a more complete picture, further studies in this area with larger patient and microRNA samples should be performed.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

AGE-RELATED MORPHO-FUNCTIONAL CHANGES IN RATS' PANCREAS UNDER HIGH-FAT DIET-INDUCED INSULIN RESISTANCE AND ITS PHARMACOLOGICAL TREATMENT

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ABSTRACT

The aim: To determine the set of structural and functional changes in pancreatic islets (PI) of obesity-induced insulin resistant (IR) rats of different age (young and old) fed with prolonged (6 month) high-fat diet (HFD) (58% of fat) and further treatment with N-Stearoylethanolamine (NSE), a bioactive N-Acylethanolamine.

Materials and methods: Alimentary obesity-induced IR model in rats of two age groups was used to investigate the influence of age and NSE treatment on pancreas morphology (using histological, histochemical and immunohistochemical techniques) and on several biochemical parameters associated with DM onset.

Results: The NSE administration normalized pancreas morphology which was more affected in the old IR group; the signs of inflammation, edema, fibrosis and steatosis were somehow diminished and PI area became significantly increased. The amount of the A-F-positive insulocytes increased and TUNEL-positive – decreased. Compensatory hyperplasia in the affected pancreas of both age was an important indicator of NSE stimulating effect.

Conclusions: Protective effects of NSE on morpho-functional state of pancreas in HFD-induced IR rats of both age are associated not only with its anti-inflammatory, anti-oxidant and anti-dyslipidemic properties but also with activation of PI hyperplasia and β-cells compensatory mechanisms.

KEY WORDS: experimental insulin resistance, pancreatic islets, Apoptosis, aging, N-Stearoylethanolamine

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INTRODUCTION

Obesity-induced insulin resistance (IR) is tightly associated with type 2 diabetes mellitus (DM), one of the most severe world-spread multifactorial age-dependent metabolic diseases, accompanied by numerous complications. According to the International Diabetes Federation, as of 2015, there were 415 million patients on the planet, and as to forecasts for 2040 the number of people with DM will increase to 642 million, acquiring the scale of the "non-infectious" epidemic of the 21st century [1,2]. Nowadays, there has been recorded an obvious aging of the population – an increase in the number of people over 60 throughout the world. Significant prevalence of DM, as well as IR and obesity, in particular is characteristic of such age category, which makes this pathology one of the main objects of gerontological research.

With continuing efforts to explore the structural basis for diabetes onset, progressive decline of β-cells of Langerhans pancreatic islets (PI) is established as a salient feature of type 2 DM [3]. That is why the mechanisms of cell failure and death, associated with the pathogenesis of this disease, are of great imp33ortance in the study of diabetes pathophysiology in different age [4-8]. Based on molecular biological studies, β-cells death in diabetic animal models has been attributed to oxidative stress and endoplasmic reticulum (ER) stress, as well as autophagy deficits [3, 9]. Thus, it is conceivable that oxidative stress damage has the strongest impact on these cells failure [10]. Pancreatic β-cell loss by apoptosis appears to play a crucial role in the development of insulin deficiency and the onset of CD [5]. Thus its age-dependent peculiarities should be taken into account for evaluation of the disease severity, prognosis and the effectiveness of various therapeutic approaches.

Evidently, β-cells are found to be more plastic, than previously thought, pointing towards the existence of powerful survival pathways. Their potentials for transdifferentiation and reversibility should be vigorously elucidated and could be a target of the future diabetes treatment [3, 6, 11]. In a large number of studies devoted to the search for DM correction methods, there are numerous researches in the field of the therapy with minor lipids that belong to the *N*-Acylethanolamines family and mediate a wide range of biological and pathological processes by interacting with cannabinoid and noncannabinoid receptors [12,13]. Recent studies indicate that *N*-Stearoylethanolamine (NSE), a bioactive *N*-Acylethanolamine, has the membrane-stabilizing,

anti-inflammatory anti-oxidant and anti-dyslipidemic action. It is able to regulate lipid metabolism, to improve the fatty acid imbalance and normalize the phospholipid levels. Such NSE-induced changes were associated with a normalization of plasma triglyceride content, considerable decrease of insulin and index HOMA-IR level in rats under IR conditions [12].

All this determines the need to investigate morpho-functional background of PI in the modeling of obesity-induced IR and its correction with NSE, taking into account the age-dependent features of these processes.

THE AIM

The purpose of the research – to study the structural, functional and morphometric features, as well as the intensity of apoptosis, in pancreatic islets of obesity-induced insulin resistant rats of different age (young and old) fed with prolonged (6 month) high-fat diet (HFD) (58% of fat) and further treatment with *N*-Stearoylethanolamine (NSE), a bioactive *N*-acylethanolamine.

MATERIALS AND METHODS

Animal Model and Treatment. The study was carried out on male Sprague–Dawley rats of two age groups – young (4 mo at the beginning and 10 mo at the end of the experiment) and old (16 and 22 mo, respectively). All the experiments were carried out according to the existing bioethical norms of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986), as well as article 26 of the Law of Ukraine "On the Protection of Animals against Cruelty" (No. 3447-IV, 21.02.2006). Rats were housed in standard cages with free access to food and water.

Obesity-induced IR was attained by feeding a prolonged HFD (58 % fat:23% proteins:10 % carbohydrates for 6 months) as described earlier [12]. The amount of lipids in the diet was increased by adding of lard to the pellet diet, which contained a high level of palmitic (24 % of total fatty acids (FA)) and stearic (28 % of total acids) acids. The HFD–FA composition was at a ratio of 55 % saturated (SFA) to 45 % unsaturated FA (USFA). Control rats were on normal pellet diet (4 % fat:23 % proteins:65 % carbohydrates) with SFA/USFA ratio 38 %/62 %, respectively.

Six months after HFD period, we conducted the oral glucose tolerance test [12, 13]. The rats of each age group (young and old) with impaired glucose tolerance (the level of blood glucose within 150 min after the oral glucose administration was higher than 5 mmol/l) were selected and divided randomly into two groups: IR (young – n =16; old – n =12) and IR + NSE (young – n = 15; old – n=12). Control rats were further subdivided into control (young – n =4; old – n =3) and NSE (young – n =3; old – n =3) groups. Animals in NSE and IR + NSE groups received the water suspension of NSE at the dose of 50 mg body weight kg^{-1} per os for 2 weeks. This particular dose of NSE has been chosen as an optimal reacting dose for the biological effect investigations [12, 13]. At the end of the experiments, the rats were decapitated under Nembutal anesthesia (50 mg/kg body weight).

Biochemical Analysis. Blood glucose levels were controlled using glucometer Rightest GM-110 (Bionime GmbH, Switzerland). The establishment of IR was confirmed based on the results of fasting plasma insulin levels (measured by Rat Insulin ELISA kit, Thermo Fisher Scientific, USA) and HOMA-IR (homeostatic model assessment – insulin resistance) value [calculated by fasting insulin (μIU/mL) x fasting glucose (mmol/L)/22.5].

Histological Examination. The pancreas was immediately removed and fixed in Bouin's solution. After dehydration the tissue samples were processed for the embedding in Paraplast® (type 6, Richard-Allan Scientific, USA). Serial sections (5 μm) were cut on a rotation microtome HM 325 (Microm, Germany) and then stained with hematoxylin and eosin (H & E) and Gomori's Aldehyde-Fuchsin (A-F) for detection of secretory granules in β-cells. The obtained histological specimens were studied and photographed at x40, x100, x200 and x400 magnification using a BX51 microscope and DP-Soft 3.2 software (*Olympus*, Japan). In all studied groups of experimental animals (control, IR, IR+NSE in two ages) the following morphometric parameters were estimated: the area of PI, their number per 1 mm^2 of the pancreas sections, the specific volume of endocrine tissue in the total volume of pancreas; specific volume of A-F-positive β-cells in the total volume of PI.

TUNEL-staining. Apoptosis was detected using TUNEL assay with ApopTag® Plus Peroxidase In Situ Apoptosis Detection Kit (*Chemicon*, USA). TUNEL staining was performed according to the protocol provided with the kit. The apoptotic index (AI) was calculated as the number of TUNEL-positive cells per 1 mm^2 of the PI section area.

Statistical Analysis. Statistical processing of the morphometric study data was performed using the Statistica 13 and GraphPad Prism 8 programs. Variational raws of data were preliminary checked to fit normal distribution, using for this purpose analysis of frequencies, and Lilliefors and Shapiro-Wilk test criteria. The obtained data were presented as mean value (M) and its standard error (SE), as well as minimal and maximal values, median (Me) and 95% of the confidence interval [95% CI]. Estimation of the statistic hypotheses during comparison of two independent groups was performed using Mann Whitney U and Kolmogorov-Smirnov criteria at α=0.05.

RESULTS

BIOCHEMICAL AND PHYSIOLOGICAL RESULTS

Throughout the experiments, the rats were gaining weight gradually. On the 24th week, the average weight of HFD rats was higher (due to visceral fat) in comparison with control rats. The obesity was more prominent in the old IR rats compared to the young that reflects the age differences in the rate of their metabolism. There was an elevation in fast-

Table I. Body weight and several biochemical parameters in the control and treated rat groups (M±SE)

	Young				Old			
Parameter	**Control**	**C+NSE**	**IR**	**IR+NSE**	**Control**	**C+NSE**	**IR**	**IR+NSE**
Body weight (g)	479.0± 24.91	506.33± 36.29	500.0± 11.50	509.81± 16.74	480.20± 19.21	489.33± 15.40	577.0± 38.29	611.2± 29.58
Blood glucose (mmol/L)	3.70± 0.19	3.73± 0.09	5.94± 0.24	4.93± 0.09	4.63± 0.32	4.90± 0.42	5.93± 0.19	5.39± 0.34
Serum insulin (μIU/mL)	0.57± 0.04	1.46± 0.08	2.45± 0.60	1.97± 0.35	2.86± 0.93	3.45± 0.08	4.24± 1.43	2.47± 0.45
HOMA	0.09	0.24	0.65	0.43	0.59	0.75	1.12	0.59

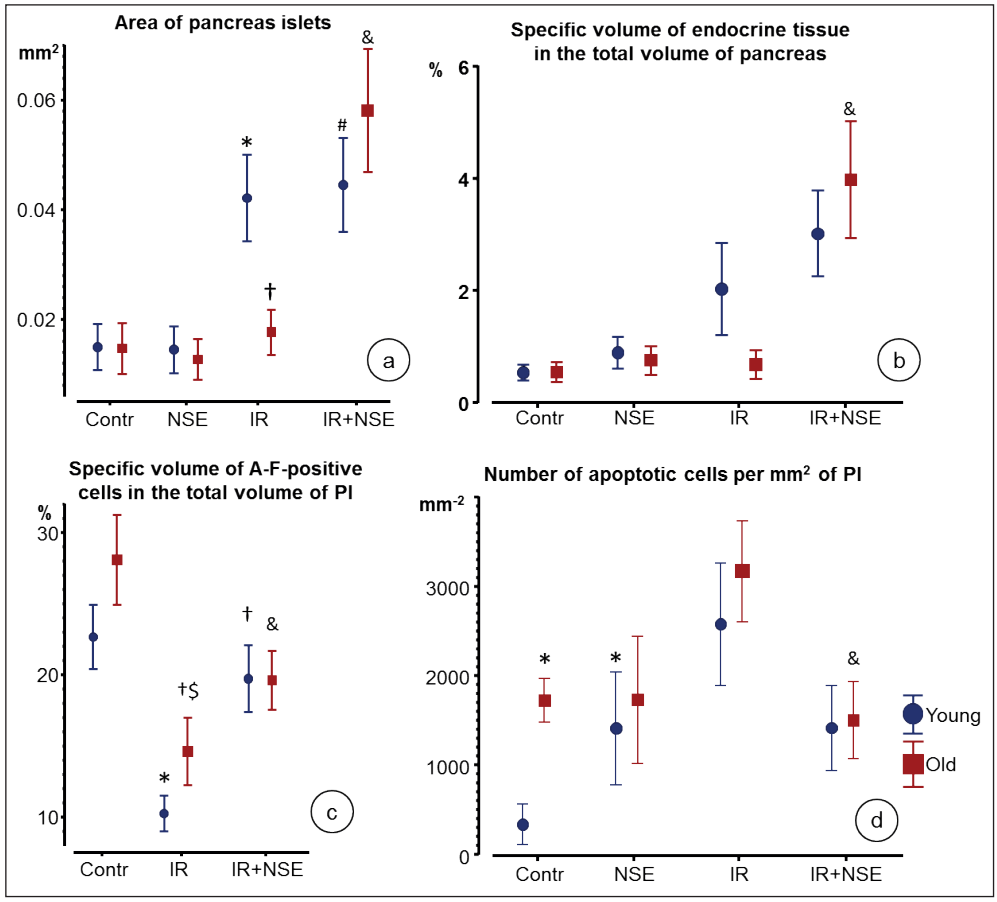

Fig 1. PI area (a), specific volume of endocrine tissue in the total volume of pancreas (b), specific volume of A-F-positive cells in the total volume of PI (c), number of TUNEL-positive cells per mm2 of PI (d) of young and old rats of various experimental groups (Control, NSE, IR, IR+NSE); mean with 95% CI on a), c), d), mean with SE on b); $P < 0.05$: * – vs C-young, # – vs C-y+NSE, † – vs IR-young, $ – vs C-old, & – vs IR-old.

ing blood glucose and serum insulin in HFD rats of both age. NSE treatment somehow normalized these values – in the young rats to a greater extent than in the old (Table 1).

HISTOLOGICAL FINDINGS

Morphological examination of the control animals' pancreata revealed its typical architecture in both age groups. Endocrine cells formed aggregates (islets) of various sizes and shape, more often small, ellipsoid or spherical. The PI epithelial cells were separated by a network of anastomosing capillaries. The morphometric parameters (the PI area and the specific volume of PI in the total volume of the pancreatic tissue) did not display significant age-related differences (Fig.1 a, b). However, in the old control some dystrophic changes of insulocytes, small accumulations of macrophages in peripheral connective tissue, as well as islet vessel dilatation, were demonstrated.

The number of insulocytes with A-F-positive secretory granules in the cytoplasm (the indicator of PI functional activity) differed a little in the young and old control animals, although its spread was significantly larger in the old, which reflects the heterogeneity of the morphological features that is generally characteristic of aging (Fig. 1 c).

Obesity-induced IR rats demonstrated severely disorganized PI architecture in both age groups. The contour of the islets and their shape became irregular (Fig. 2a). PI appeared to be larger in size and quantitatively more numerous compared with normal-diet controls in the young group whereas these values didn't change significantly in the old animals (Fig.1 a, b). At the same time the number of insulocytes with structural disarrangements and dys-

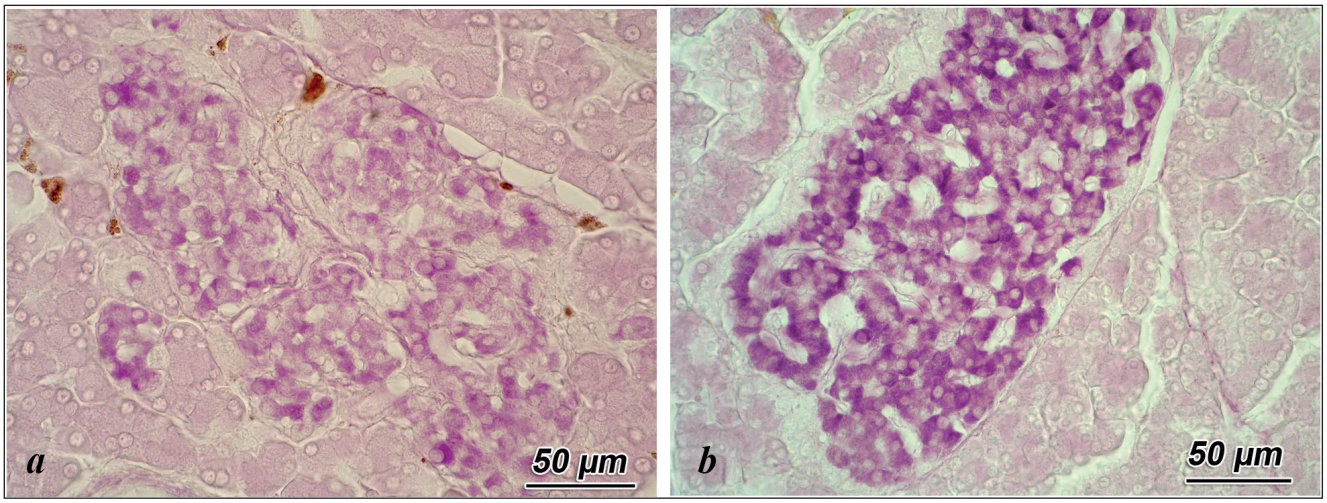

Fig 2. PI of young IR (a) and IP+NSE (b) rats with low and high histochemical activity of insulocytes (Aldehyde-Fuchsin staining).

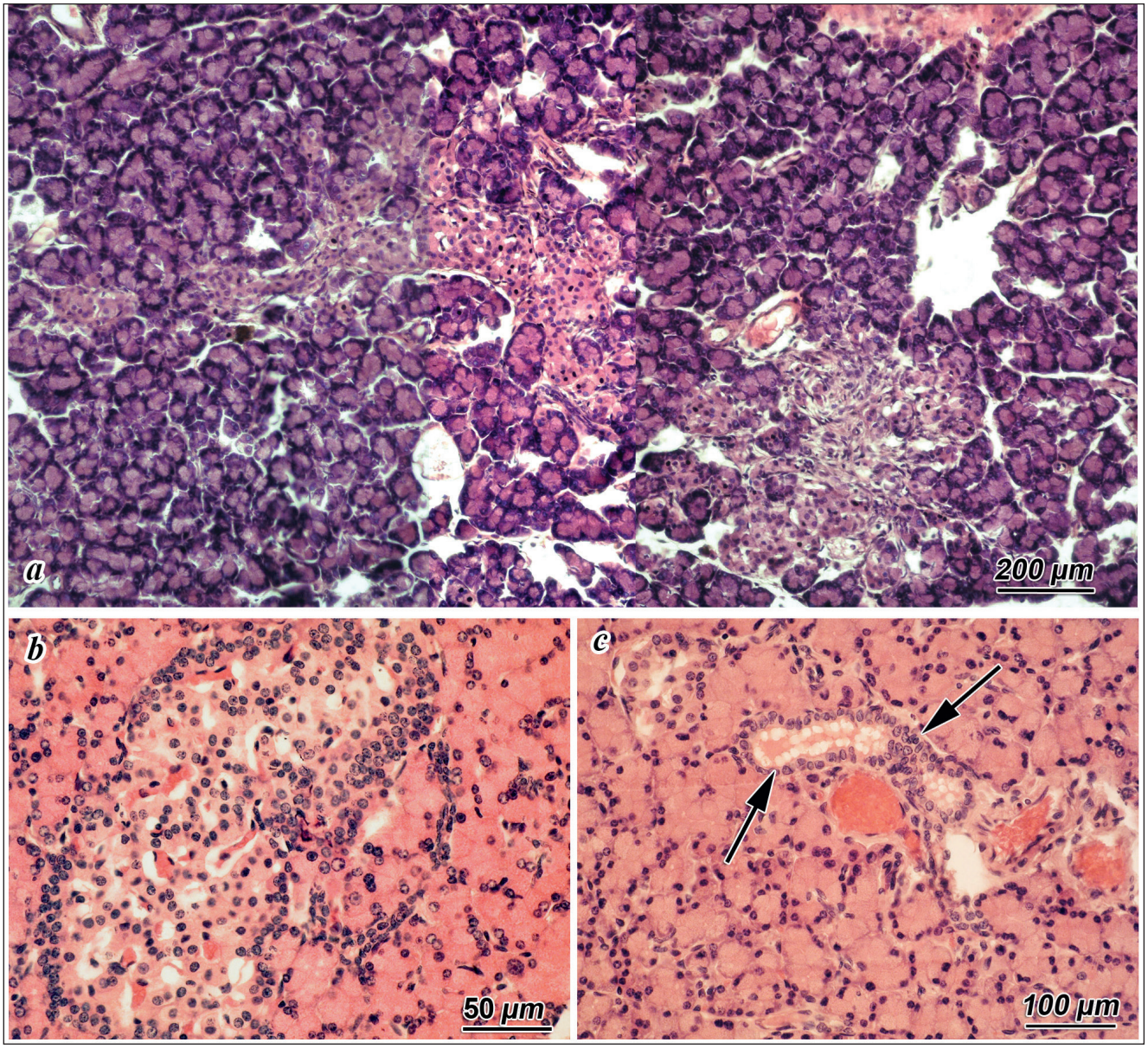

Fig 3. Pancreatic sections of old (a) and young (b, c) rats of IR+NSE groups. Irregular shape and hyperplasia of PI (a). Hyperplasia of PI cells (b) and duct epithelium (arrows) (c). (H & E).

trophic changes, as well as edema of their cytoplasm, was much higher in the old IR rats compared to young. The damaged islets were also more numerous in the old age group. Pronounced islet vessel dilatation was observed as well (Fig.2 a). Moreover, the presence of inflammatory infiltration as well as fibrosis were found in PI of the predominantly old animals. Besides it was possible to observe fatty replacement of the exocrine pancreas in both age groups (more pronounced in the old) which is the manifestation of pancreas steatosis in long-term HFD treated animals.

While inducing IR, the level of functional activity of the insulocytes decreased sharply in the rats of both age, reflecting the deeper destructive, dystrophic and atrophic processes in the PI of obesity-induced IR animals. The A-F-positive area in affected pancreas was significantly smaller than in normal conditions (Fig. 1 c). There were insulocytes that had very few secretory A-F-positive granules in their cytoplasm that corresponds to reduced number of the functionally active β-cells that is resulting in decrease of insulin secretion (Fig. 2 a).

Obesity-induced IR rats treated with NSE showed pronounced changes of PI architecture in both age groups. Though their morphology was normalized to a certain extent, the quantitative parameters of PI changed evidently (Fig. 1 a, b). Islet size was significantly increased in groups IR+NSE compared both to IR and control groups (Fig. 3 a). The islets became both enlarged and much more irregular in shape; sometimes they lost the oval-like shape and spread along the pancreas. Such PI enlargement was associated with islet cells hyperplasia that was observed not only in IR+NSE groups but also in control animals that received NSE (Fig.3 b). Moreover, there has been shown the hyperplasia and hypertrophy of duct wall epithelium which is considered to be primitive precursor cells and a primordium of islets (Fig.3 c). The level of inflammatory infiltration was reduced in PI of IR+NSE young animals more then in old ones and the islet vessel dilatation was also a little bit declined in these groups.

In IR+NSE groups the amount of insulocytes with A-F-positive secretory granules in their cytoplasm increased compared to HFD treated animals that did not receive NSE, though these parameters did not gain the control values (Fig.1 c, 2 b).

TUNEL IMMUNOHISTOCHEMISTRY

Apoptotic cells were detected by TUNEL assay and appeared as brown-stained. The level of apoptosis in PI was significantly higher in old control animals compared to young due to age-related changes in the gland (Fig. 1 d).

While inducing IR, AI in PI increased significantly, especially in the old rats, being correlated with much deeper structural damage of the pancreas, that was noted in this age group. The IR+NSE groups showed a decrease of AI in PI compared to the animals that did not receive NSE treatment (Fig.1 d). That is an objective criterion for the effectiveness of this kind of therapy.

DISCUSSION

The age aspect of the CD pathomorphosis acquires special attention not only due to an obvious increase in the prevalence of this pathology with age, but also because a number of its manifestations, in terms of their morphological characteristics, resemble the age changes typical for normal physiological aging. Evidently, the pre-existing age-related morphological and functional alterations in various organs contribute to the development of deeper diabetic lesions, which has been confirmed by our previous data [7,8]. Our study of the corrective effects of gene therapy (with PEI-pDNA complex containing the human preproinsulin gene) on the morphological and functional characteristics of various mice organs, including pancreas, at streptozotocin-induced DM showed its different efficacy in different age: high effectiveness in the young animals and a slight effect, its absence, or even additional deteriorations (e.g. insulitis) in the old ones. So the effects of gene therapy application in this mice model appeared to be age-dependent.

In this study, performed on the rat model of HFD-induced obesity, IR and subsequent type 2 diabetes, we did not determine demonstrative and pronounced age-related differences in the effectiveness of NSE treatment which is considered to be a novel anti-diabetic drug [12, 13]. Thus, pharmacological treatment with NSE of obesity-induced IR rats appeared to be rather effective in both ages despite of definite age-related distinctions in their reaction to the long-term HFD treatment, as has been shown in this study.

The NSE administration normalized to a certain degree pancreas morphology which was more affected in the old IR group; the signs of inflammation, edema, fibrosis and steatosis were somehow diminished. The amount of the A-F-positive insulocytes increased and TUNEL-positive – decreased. In addition, the NSE treatment stimulated the development of compensatory hyperplasia in the affected pancreas, which is an important indicator of its protective effects on the IR animals' pancreas of both age, supporting the role for divergent mechanisms in controlling adaptation to metabolic demand.

Despite the existence of contradictory data, the viewpoints about the worsening of β-cells function in aging, the decrease in their proliferative activity and the increase in the apoptosis level prevail in the literature [3, 6]. At the same time, numerous studies performed in animal models, as well as in patients with DM, associated with the study of the mechanisms of injury, death, regeneration and adaptation of β-cells, indicate that they have greater plasticity than previously thought, which allows to consider them to be a perspective target for finding new ways for DM treatment [3, 6, 11]. The potential mechanisms of β-cell regeneration, including their self-replication, neogenesis from non-β-cell precursors and transdifferentiation from a-cells, as well as tissue stem cells, are discussed by the different authors [3, 14,15]. The ability of the pancreas to regenerate mature β-cells is explored in pathological conditions, including diabetes and inflammatory injury.

Our findings of the pronounced PI compensatory hyperplasia as well as the duct epithelium activation after NSE

administration both in the intact and in the HFD-induced IR rats of different age revealed the strong capacity for islets regeneration that is preserved even in the old. Such results concerning impressive plasticity of the endocrine pancreas of adult and even old animals under pharmacological activation are especially important, since they can help to clarify the characteristic features of corrective effects at this disease in old age.

CONCLUSIONS

Our data obtained by biochemical, histological, histochemical and immunohistochemical techniques confirmed the development of PI histopathological alterations in the obesity-induced IR rats of two age groups fed with prolonged (6 mo) HFD, which were more pronounced in the old animals. The NSE administration normalized pancreas morphology: the signs of inflammation, edema, fibrosis and steatosis were somehow diminished. The amount of the functionally active A-F-positive insulocytes increased and TUNEL-positive cells, involved in apoptosis process – decreased. This serves an objective criterion for the effectiveness of such kind of therapy.

Our findings of the pronounced PI compensatory hyperplasia as well as the duct epithelium activation after NSE administration both in the intact and in the HFD-induced IR rats of different age revealed the strong capacity for islets regeneration, enlargement and activation that is preserved even in the old animals.

The protective effect of NSE on morpho-functional state of pancreas in HFD-induced IR rats of different age can be associated not only with its anti-inflammatory, anti-oxidant and anti-dyslipidemic properties but also with its capacity to activate PI hyperplasia and β-cells compensatory mechanisms.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

MORPHOLOGICAL FEATURES OF RATS' HEARTS AFTER INTRAFETAL INJECTION OF DEXAMETHASONE

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ABSTRACT

The aim: Is to study the morphological features of rats' hearts after prenatal administration of glucocorticoids.

Materials and methods: In this study we used histological, immunohistochemical, electron-microscopic and statistical research methods.

Results: It is found that at 30th day after birth in rats after intrafetal introduction of dexamethasone in myocardium a relative area occupied by arterial vessels is significantly smaller in comparison with control. Absolute and relative number of Ki-67$^+$-cardiomyocytes in the myocardium of experimental rats is reduced throughout the second week after birth and is significantly less compared to the control group. In the nuclei of cardiomyocytes of experimental rats is rendered the greater amount of heterochromatin in comparison with cardiomyocytes of the control group where euchromatin prevails.

Conclusions: After intrafetal injection of dexametazone changes in dynamics and significantly smaller index of relative area occupied by arterial vessels in ventricular myocardium at the 30th day after birth are observed; the absolute and relative number of Ki-67$^+$ -cardiomyocytes in myocardium decreases during the second week after birth and is significantly lower compared to the control group; in the nuclei of cardiomyocytes of experimental rats a greater amount of heterochromatin is visualized, and in cardiomyocytes of the control group – euchromatin.

KEY WORDS: cardiomyocytes, heart, dexamethasone

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INTRODUCTION

The study of the causes and mechanisms of cardiovascular system diseases development is one of the topical issues of modern experimental medicine. The share of cardiovascular system pathology in the structure of mortality from non-communicable causes in Ukraine is about 67 %, and according to this criterion takes one of the first places in the world [1].

In the XX century, the English epidemiologist David Barker, formulated a theory according to which the conditions of intranatal development contribute to the formation of health and diseases in future [2]. In particular, it was found that adverse conditions in utero could program cardiovascular diseases in adulthood [3]. Morphological basis for the development of these conditions may be a syndrome of unclassified connective tissue dysplasia, which is manifested by disorganization of connective tissue fibers that gradually lead to decrease of adaptive possibilities of the body in future [4]. At that time adverse factors, can influence the morphogenesis of organs and systems during gestation, there may be drugs that women take during pregnancy, in particular, a synthetic glucocorticoid hormone dexamethasone.

According to "European guidelines for the management of premature newborns with respiratory distress syndrome" (2019), prenatal corticosteroid therapy is recommended for all women with threatened preterm labor before the 34th week of pregnancy [5]. In particular, in Ukraine, according to the current clinical Protocol on obstetric care "Premature birth" (the Ministry of health Order № 624 dated 03.11.2008), in case of the premature birth threat, the prevention of respiratory distress syndrome of the fetus from 24th to 34th week by intramuscular injection of dexamethasone is conducted. However, the appropriateness of the appointment of glucocorticoid hormones to pregnant women in the third trimester is a controversial issue according to potential negative long-term consequences [6]. Previously it is shown that intranatal introduction of glucocorticoids influences on different processes of connective tissue development: it is settled that in rats after glucocorticoid introduction in fetal period more earlier as compared to control thinning of articular cartilage, diminishing of relative area occupied by fibres in articular capsule, their disorganization, shortening and diminishing of depth of penetration in the matrix of articular cartilage of the joined bones, more later as compared to control appearance of elastic fibres in articular capsule are defined [7]; a significant increase of the relative area occupied by intertrabecular lacunas in subchondral bone compared to control is revealed [8].

In particular, there is a connection between prenatal exposure to synthetic glucocorticoids and an increased risk of developing cardiovascular disease further in adulthood [9].

In rodents physiological increase in the synthesis of endogenous glucocorticoids by mothers' adrenal glands starts at the 15th day of pregnancy, in humans – during the last week before birth. On the other hand, the level of placental 11-beta-hydroxysteroiddehydrohenase (11β-HSD) decreases during the last

week of pregnancy both in rodents and humans, which leads to the passage of maternal glucocorticoids to the fetus by the end of pregnancy and provides functional maturation of organs and systems [10]. However, dexamethasone is not metabolized by 11β-HSD, and it can pass freely through the placental barrier during the embryonic period from mother to fetus [11]. In experiments with animals it was found that the effect of glucocorticoids in late pregnancy led to structural maturation of the fetal heart by increased proliferation of cardiomyocytes at the 19-th and 21-th day of fetal development [12].

However, the morphological features of rats' hearts after prenatal administration of glucocorticoids are still need to be reviewed.

THE AIM

The aim of the research is to study the morphological features of rats' hearts after intrafetal injection of dexamethasone.

MATERIALS AND METHODS

The object of the study was 144 heart of white laboratory rats, which were divided into 3 groups: the 1st – group of intact animals; the 2nd group consisted of experimental animals, each of which at the 18th day of fetal development was injected by 0.05 ml of dexamethasone; the 3rd group of control rats, which were injected by 0.05 ml of 0.9% NaCl at the 18th day of intranatal development. The formation of a control group of rats is due to the need to eliminate the influence of surgical intervention in the prenatal period on the obtained changes in the experimental group.

Animals were contained in standard conditions of vivarium according to "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 18.03.86 G.) and the Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty. Morphological structure of heart was examined at days 1st, 3rd, 5th, 9th, 14th, 21th, 30th and 45th after birth. Fixation of histological material was carried out in 10% neutral formaldehyde. Immunehistochemical, histological methods, method of electron microscopy and statistic methods were used in the work.

Serial histological sections 4 μm in thickness after dewaxing were stained by hematoxylin and eosin according to standard techniques, all samples were embedded in the balm. Photos of histological samples were conducted using CarlZeiss software (AxioVision 4.8). Calculation of the relative area that is occupied by the arterial vessels in ventricles of rats was performed using the electronic program ImageJ with an overlay of masks. Percentage values were obtained as the ratio of the number of pixels that corresponded to the studied structures that are specifically colored, to the total number of pixels in the digital image of the sample, with the data obtained in the area of 9000 μm2. For electron microscopy, ultra-thin sections of myocardial fragments of the ventricles of the heart were made, they were counterstained with lead citrate according to the Reynolds method and examined in an electron microscope PEM-100-01 at an accelerating voltage of 75 kV. To detect proliferating cardiomyocytes immunohistochemical studies were performed using Ki-67: sc-23900 mouse monoclonal antibody (Santa Cruz Biotechnology, inc.). Immunohistochemical reaction was visualized using an UltraVision Quanto HRP + DAB System from Thermo Scientific, followed by photodocumentation and processing in ImageJ. The amount of Ki-67 + -cardiomyocytes was counted per 105 μm2 of the ventricles of the heart of animals.

Analysis of the obtained results was conducted by means of statistical methods with the use of computer license program «Statistica for Windows 13» (StatSoft Inc., № JPZ804I382130ARCN10-J). The statistical significance of the obtained differences of indicators in the comparison groups was evaluated using the Mann-Whitney U test and considered to be significant at $p < 0.05$, that is generally accepted for biological and medical researches. The numerical data of the obtained results are presented as M ± m (arithmetic mean ± standard error of the mean).

Ethnical approval. Supporting and withdrawal of animals from experiment was carried out in accordance with the requirements of the European Commission Directive (86/609/EEC), Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty.

RESULTS

The dynamics of the relative area occupied by arterial vessels in myocardium of intact and control animals changes wavy with a maximum value at the 30th day – 6,92 ± 2,21 and 7,05 ± 1,67%, respectively (Table I).

In animals of intact group, this index increases gradually just from birth up to the 30th day multiplying in 5.25 times with maximum increase at the fifth day (in 2.07 times), and then reduces by 40.75 %. The relative area of arterial vessels in myocardium of control animals runs up in 5 times by the end of the first month of observation, later on at 45th day it decreases by 36.59 %. The value of this index in intakt and control groups of animals were not significantly different in all periods of observation.

Dynamics of the relative area occupied by arterial vessels in myocardium of experimental animals after intrauterine injection of dexamethasone differs from data of intact and control groups: maximum value (4,28±1,26 %) is revealed at the 14th day after birth. Overall, the value of this index in experimental group of animals increases in 1.95 times during the first 2 weeks after birth. Then it gradually reduces up to 45 days by 24.3 %, and at the 30th day of life, the relative area occupied by arteries in myocardium of experimental animals is significantly lower compared to the values of the control group (3.34 a±1,18 and 7.05±1,67 %, respectively).

In ventricular myocardium of rats' heart of all groups of animals we define cardiomyocytes which express the Ki-67 antigen on the membrane of their nuclei (Fig. 1).

At day 9th after birth in experimental animals, the absolute number of Ki-67+ cardiomyocytes in myocardium was 200.0 ± 12.8 cells per 10^5 μm^2 that index was significantly lower in comparison with control – 248.0 ± 14.4. In the

Table I. The relative area occupied by arterial vessels in myocardium of animals (M±m, %)

	Intact group of animals	Experimental group of animals	Control group of animals
1st	1,32±0,49	2,19±1,13	1,41±0,49
3rd	1,77±0,54	2,73±0,75	1,98±0,74
5th	3,68±1,09	3,19±1,04	3,30±0,92
9th	4,51±1,75	3,70±0,97	4,44±1,25
14th	4,77±1,61	4,28±1,26	5,04±1,22
21st	5,37±1,36	3,79±1,20	5,48±1,84
30th	6,92±2,21	3,34±1,18*	7,05±1,67
45th	4,10±1,18	3,24±1,18	4,47±0,83

* –result is reliable in relation to control group (p<0,05).

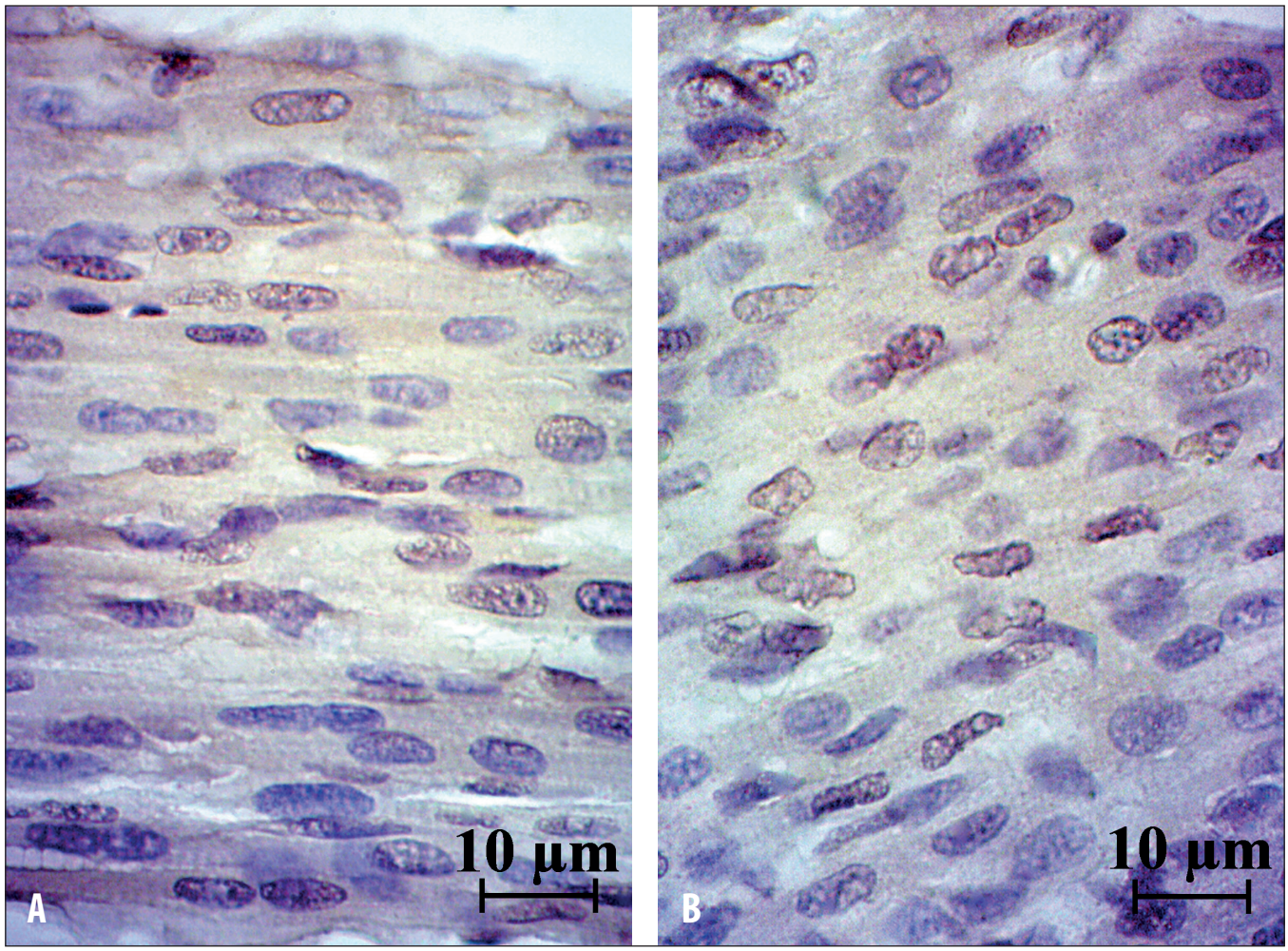

Fig 1. Ki-67+-cardiomyocytes in myocardium of left ventricle of control rat. X 1000. A) the 9th day after birth, b) the 14th day after birth.

group animals after intrafetal injection of dexamethasone the relative number of Ki-67 + cardiomyocytes to the total number of cells in the area of view was also significantly lower compared to the data of the control group (18.4 ± 1.2 and 26.4 ± 1.2%, respectively).

At day 14th after birth the absolute number of Ki-67+ cardiomyocytes in the group of animals after intrafetal injection of dexamethasone decreases by 36.8%, compared to the previous observation period and is 126.4 ± 3.2 cells per 10^5 µm^2 and is significantly lower than in control rats (297.1 ± 22.4 cells per 10^5 µm^2). The relative number of Ki-67 + -cardiomyocytes in the experimental group was also significantly lower compared to the values of the control group (16.7 ± 1.0% and 26.2 ± 1.5%, respectively).

Electron microscopy revealed that the greater amount of heterochromatin is visualized in the nuclei of cardiomyocytes of rats after intrafetal injection of dexamethasone, and in cardiomyocytes of animals of the control group euchromatin prevails (Fig. 2).

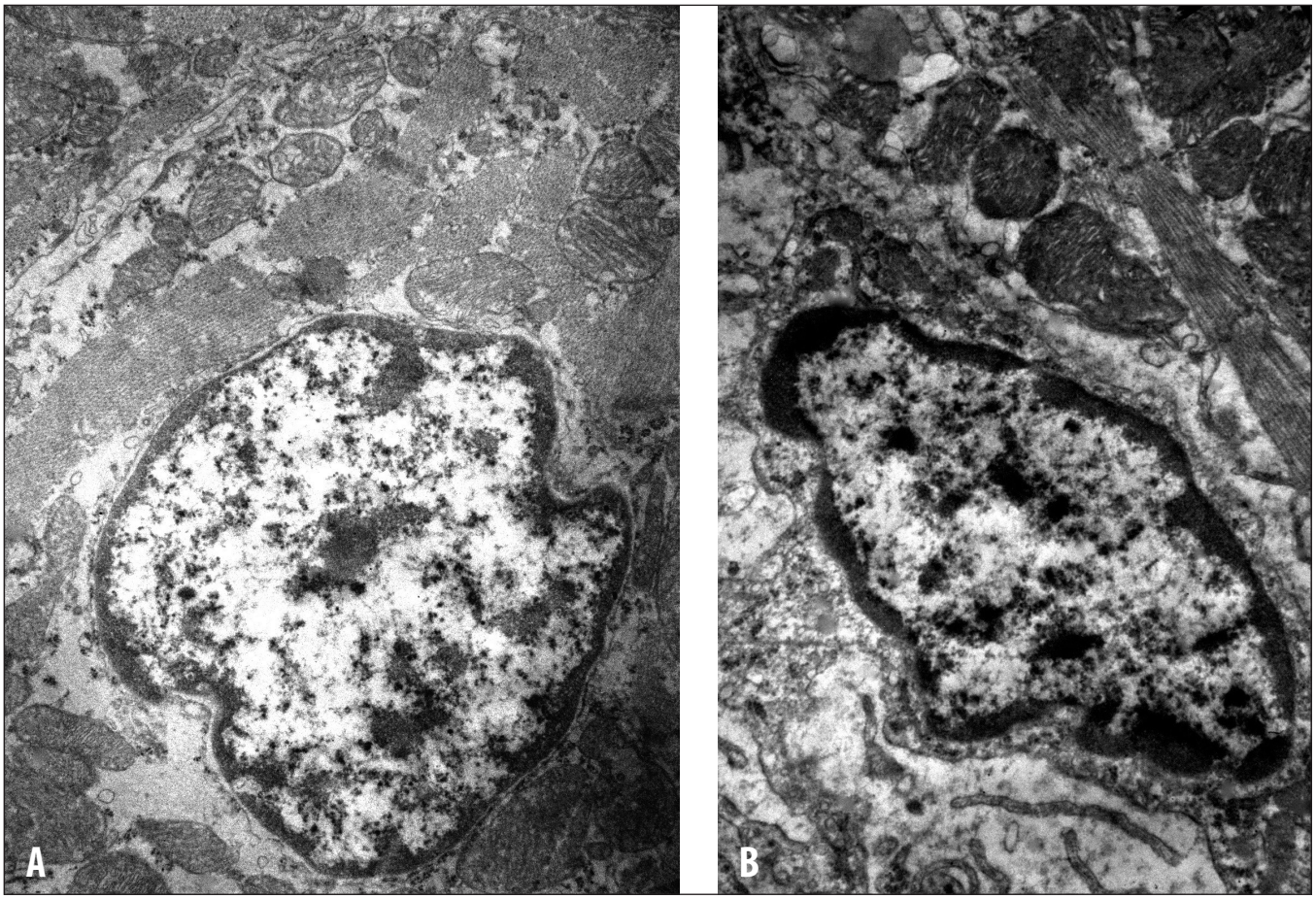

Fig 2. Myocardium of rats' left ventricle at day 14th after birth. Nucleus of cardiomyocyte. X 7500. A) control rat; b) rat after intrafetal injection of dexamethasone.

DISCUSSION

Dynamics of the relative area occupied by arterial vessels in the ventricular myocardium of intact and control rats, within 45 days of postnatal life, consistent with the views of Kok Wah Hew (2003), postulating that morphogenesis of arterial vessels in rats lasts for a month after birth [13]. After intrafetal injection of dexamethasone a premature maturation of the vascular component of the myocardium is established proved by changing in ratio towards earlier cessation of increasing of relative area occupied by arteries. Such changes are consistent with the literature data according the ability of dexamethasone to inhibit proliferation of smooth muscle musculature of the arteries, which leads to their premature maturation [14]. Together with previously obtained data on the reduction of the thickness arterial wall complex intima-media in myocardium after intrafetal injection of dexamethasone, these changes can lead to vascular dysfunction of heart in future.

Previously, we have described changes in nuclear-cytoplasmic relations in myocardium of rats after intrafetal injection of dexamethasone. [15]. It is found that in the experimental group the number of cardiomyocytes' nuclei intensively increases during the first five days after birth, after that myocardium thickens due to increased sizes of cardiomyocytes not due to increased their number. Obtained results of the study are consistent with earlier data on the participation of dexamethasone in premature maturation of the heart. The number of Ki-67$^+$-cardiomyocytes in myocardium of intact and control animals increases from 9th up to 14th day after birth and corresponds to the period of terminal cardiomyocytes' binucleation, which in rats lasts for two weeks after birth. The decrease in number of Ki-67$^+$-cardiomyocytes in the heart of rats after intrafetal injection of dexamethasone during the second week after birth indicates a premature decrease of proliferative activity of cardiomyocytes, i.e., early maturation of myocardium. Transmission electron microscopic study of cardiomyocytes' nuclei of ventricular myocardium confirms this assumption. In fact, revealed increasing amount of heterochromatin at day 14th after birth in experimental group, while in the nuclei of cardiomyocytes of control animals a greater amount of euchromatin is revealed. That is, in rats after intrafetal injection of dexametazone at the fourteenth day after birth cardiomyocytes are incapable of mitotic division, whereas in control group this possibility still remains. Thus, dexamethasone leads to appearance of premature binuclear cardiomyocytes, which potentially reduces the number of functional units of the heart, which in future will have more intense growth to develop a sufficient power of contraction due to the greater load on exact cells [16]. This may lead to compensatory hypertrophy and reduced adaptive capacity of myocardium, which in future will increase the risk of coronary heart disease. All this indicates the ability of prenatal dexamethasone to program cardiovascular disease in adulthood.

CONCLUSIONS

1. After intrafetal injection of dexametazone changes in dynamics and significantly smaller index of relative area occupied by arterial vessels in ventricular myocardium at the 30th day after birth are observed.
2. After intrafetal injection of dexametazone the absolute and relative number of Ki-67 $^{+}$ – cardiomyocytes in myocardium decreases during the second week after birth and is significantly lower compared to the control group.
3. In the nuclei of cardiomyocytes of the rats' heart after intrafetal injection of dexametazone, a greater amount of heterochromatin is visualized, and in cardiomyocytes of the control group – euchromatin.
4. The revealed changes indicate premature maturation of cardiomyocytes after intrafetal injection of dexametazone, which reduces their number in future life.

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ORIGINAL ARTICLE

BIOFILM FORMING ACTIVITY OF NON-FERMENTING GRAM-NEGATIVE BACTERIA

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ABSTRACT

The aim: To study the influence of chemical, physical factors on the biofilm forming activity of P. aeruginosa, A. baumannii.

Materials and methods: Biofilm forming activity of P. aeruginosa (10 isolates) and A. baumannii (10 isolates) was studied in nutrient media of different composition. There was used the method in 96-well crystalline violet staining plates with spectrophotometry (STAT FAX®4300, wavelength of 620 nm).

Results: Results showed that in standard medium (trypto-soy broth), strains of P. aeruginosa (90%) and A. baumannii (60%) obtained high biofilm forming activity. A. baumannii formed biofilms even in sterile water. Biofilm forming activity of urease positive P. aeruginosa increased in the medium with 1.0% urea. Both Acinetbacteria and Pseudomonas intensively produced their biofilms in the presence of 5% serum or sub-bacteriostatic concentrations of levofloxacin in the media. High concentrations of sodium chloride inhibited their biofilm activity.

Conclusions: Isolates of Acinetobacter and Pseudomonas obtain the protective biofilm-forming ability under such adverse environmental conditions as insufficient nutrients, high osmotic pressure, the presence of antibiotics but at high concentrations sodium chloride biofilm-formation is stimulated only in the first bacteria and suppressed in the second one.

KEY WORDS: bacteria, biofilm, levofloxacin, acinetobacteria, Pseudomonas

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INTRODUCTION

Far evolutionary and taxonomically different bacteria are united in a heterogeneous group of non-fermenting Gram-negative bacilli by a number of phenotypic features and are characterized by ubiquitous distribution. High ecological plasticity allows these bacteria to dominate in many ecological niches, as they are common inhabitants of natural reservoirs, found in soils of different geographical zones, sewage, capable of colonizing numerous plant substrates, skin and mucous membranes of animals and humans. In human surroundings, they can be found on the surface of sanitary equipment, in ventilation and humidification systems, surfaces of kitchen equipment and equipment for cleaning rooms, computer keyboard, soil of houseplants, etc. These bacteria have a selective advantage over other microflora, primarily because of their minimal nutrient requirements and natural resistance to chemical influences, including exposure to antibiotics and disinfectants [1, 2, 3].

In recent decades, the representatives of the genera *Pseudomonas* and *Acinetobacter*, which role in the etiological structure of opportunistic human infections is steadily increasing, attract special attention of specialists. Taking into account the selective benefits, favorable conditions for microorganisms of these genera exist in the hospital environment. The greatest problems of *Pseudomonas* and *Acinetbacteria* are created in the intensive care units, departments of combustological and surgical profile, where patients with secondary immunodeficiency due to severe underlying disease are concentrated. The ability of these microorganisms to easily colonize the metal and polymer surfaces of medical equipment and tools has expanded the list of entrance gates for them because of increasing the number of invasive manipulations in the process of improving medical technologies [4, 5, 6, 7].

Alarming statistics on the increase in the number of infectious lesions, associated with the provision of medical care due to antibiotic-resistant variants of *P. aeruginosa* and *A. baumannii*, have forced in February 2017, the World Health Organization to put two types of bacteria on the list of "priority pathogens", which pose the greatest threat to human health [8]. The most dangerous clinical characteristic of *Pseudomonas* and *Acinetbacter* is the high level of natural and acquired resistance to antibiotics. The presence of resistance to many antibiotics is determined even in the "wild" strains of these bacteria, isolated from the environment not contaminated with human activities [4, 9, 10].

Hospital strains are usually characterized by the simultaneous presence of many mechanisms of resistance to β-lactam antibiotics, aminoglycosides, fluoroquinolones, amphenicols, tetracyclines. Since the 1990s, strains of *Acinetobacter* and *Pseudomonas*, resistant to carbapenems and colistin have been recorded and the rate of isolation of such isolates has been steadily increasing. So in surgical hospitals of Ukraine for the

period from 2011 to 2015 the release rate of carbapenem-resistant *P.aeruginosa* strains increased by 67.4%. Similar trends are found in other countries. There are no effective methods of therapeutic management for patients with infectious processes caused by pan-resistant to antibiotics bacterial strains. This leads to high mortality rates and to significant socio-economic losses [9, 10, 11, 12].

In *P. aeruginosa* and *A. baumannii* there is proved the ability to survive in the presence of high concentrations of antibiotics, antiseptics and disinfectants, due to the presence of enzymatic and efflux mechanisms of action on antibiotic molecules and formation of biofilms at the locus of lesions. After all, these types of bacteria are characterized by the highest level of biofilm forming activity, in comparison with other bacterial pathogens. Bacteria biofilms are a complex community of bacterial cells, that are fixed on any surface and united by an extracellular polymer matrix. Biofilms have ordered multilayered topography and cytoarchitectonics, but individual cells in their composition differ in metabolic activity and can be functionally differentiated. In fact, bacterial biofilms are a form of extracellular organization of bacteria, that enhances the population's ability to survive. As a part of biofilms, bacteria obtain the resistance to aggressive environmental factors [13, 14, 15].

The structure of bacterial biofilms and the dynamics of their formation have been studied in detail at the molecular level. Many studies are devoted to the comparative evaluation of the biological properties of bacterial cells in the composition of biofilms and planktonic forms. However, in the scientific literature there is insufficient data about the influence of external factors on the biofilm process [16].

Understanding of the factors, influencing the intensity of biofilm formation by *Acinetobacter* and *Pseudomonas*, will help reliably predict the likelihood of the formation of highly resistant forms of pathogens inflammatory processes in clinical settings, and make this important biological process manageable.

THE AIM

The aim – to study the effect of osmotic pressure and the presence of certain organic substances, antibiotics in a nutrient medium on the intensity of film formation of *P. aeruginosa*, *A. baumannii.*

MATERIALS AND METHODS

In the research there were used 10 clinical strains of *P. aeruginosa* and *A. baumannii*, isolated from patients, treated in burn and surgical departments of Vinnitsa medical establishments. Identification of microorganisms was performed taking into account morphological, tintorial, culture and biochemical properties. Biochemical typing of isolated strains was performed using diagnostic panels NEFERM test 24 (PLIVA – Lachema a. s. Brno, Czech Republic).

Determining the biofilm forming activity of studied strains was carried out using the standard method of analyzing bacterial biofilms in 96-well platelets with crystalline dye. The intensity of the film formation was estimated by the optical density of the studied samples of bacterial film. Cultivation of the microorganisms was carried out in tryptone soy broth (TSB) (GRASO Biotech, Poland) and "starvation" media based on sterile distilled water with the addition of inorganic and organic compounds. All spectrophotometric measurements were performed on a STAT FAX® 4300 spectrophotometer (Netherlands) at a wavelength of 620 nm. The optical density (OD) for each strain was determined in three replicates, the results averaged. A strain was considered to be positive for film-forming ability if its average OD value was greater than the meaning of optical density in negative control, which had increased by three standard deviations (SD): (OD negative control + (3 × SD negative control). The negative control OD was calculated for each tablet separately. The intensity of the film formation was evaluated by the value of the relative optical density [17].

The sensitivity of the tested strains of microorganisms to antibiotics was studied by the method of double serial dilutions of the drug in liquid nutrient medium (TSB). The artificial formation of resistance of microorganisms to antibiotics (meropenem, amikacin, levofloxacin) was performed *in vitro* by the method of passages of microorganisms in meat-peptone broth under the increasing antibiotic concentrations. For this purpose, a series of consecutive double dilutions of the antibiotic in tubes with meat-peptone broth was prepared. The test cultures of bacteria were introduced into test tubes and incubated for 24 hours at 37^0 C. After that, in a row there had been determined the tube with the maximum concentration of the antibiotic, in which there was found no bacteriostatic action of this antibiotic. The content from this tube was used as inoculum for the next passage.

RESULTS

Determination of the film-forming activity of clinical strains of genius *Pseudomonas* and *Acinetobacter* when cultured in TSB at 37^0 C demonstrated significant differences between strains. In general, the property studied was expressed more strongly in *P. aeruginosa*. Biofilm forming abilities of the tested microbial strains are presented in fig. 1. Accordingly, a black horizontal line indicates the lowest OD value above which the strain was considered positive for biofilm ability.

Of the 10 *P.aeruginosa* isolates studied, only one strain did not meet the chosen criterion. Among the tested strains of *A. baumannii*, four of them did not show any film-forming activity. In this case, the parameters of intensity (M±SD) of the formed biofilm in *P. aeruginosa* was 0.53±0.23, and it was significantly lower in *A. baumannii* (0.26±0.17). Interestingly, in the content of the studied strains of microorganisms in the absence of any nutrients (sterile water for injection), *Acinetobacter* showed higher level of biofilm forming activity, in comparison with *Pseudomonas.* The average rate of *P. aeruginosa* biofilm formation under these conditions did not differ from the negative control, and in the tested strains of *A. baumannii* was 0.094 ± 0.037. In culture media containing one of the carbohydrates, which were able to be cleaved by tested strains (accordingly to their verified enzymatic activity: glucose or galactose), significant interspecies differences were detected at 1%.

Strains with initial high rates of biofilm forming activity in the presence of carbohydrates did not undergo signif-

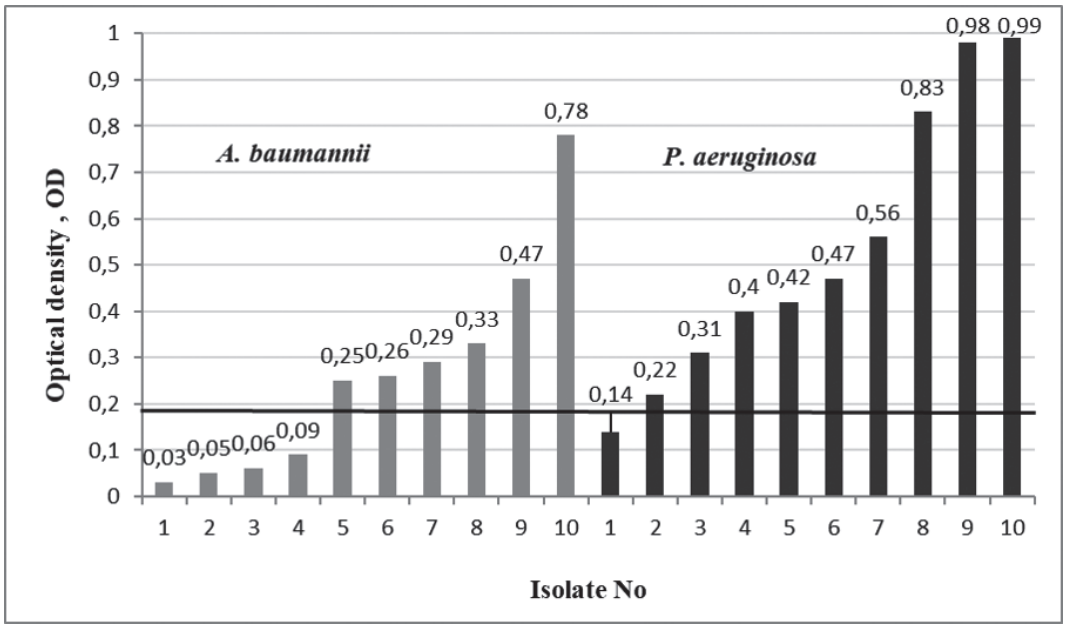

Fig. 1. *P. aeruginosa* and *A. baumannii* film-forming activity in TSB cultivation.

icant changes in this indicator. Strains without biofilm forming activity in "hungry" conditions, significantly have activated this process in the presence of carbohydrates. The intensity of biofilm formation in such strains has increased by 34 – 38% in comparison with the initial level. A similar pattern was extended to representatives of both species of non-fermenting bacteria studied. Addition of 1% of any single amino acid (lysine, leucine, arginine) into the "hungry" media did not affect the biofilm forming activity of *Pseudomonas* and *Acinetobacter* in the fasting environment. The same pattern was also proved in strains of *Pseudomonas*, which had showed the ability to cleave amino acids in the NEFERM test. As for, *Acinetobacter*, they did not alter film-forming activity in the presence of 1.0% urea in nutrient solution. Urease positive strains of *Pseudomonas* increased the film-forming intensity by an average of 52.0% to baseline. Biofilm forming activity of bacteria of both species significantly increased in the presence of 5.0 % of normal equine serum. In *Pseudomonas*, the average increase in the intensity of biofilm formation was 21.0%, and in *Acinetobacter*, it reached 67.0%.

The study of the effect of osmotic pressure on the biofilm forming activity of Gram-negative non-fermenting bacteria was performed in conditions with an media in which glucose at a concentration of 1.0% was the only source of energy and carbon. Osmotic pressure was created by adding sodium chloride in various concentrations to the medium. The average values of the intensity of biofilm formation by *Acinetobacter* and *Pseudomonas* under conditions of different osmotic pressure are illustrated in fig. 2.

There was found, that in 0.9% sodium chloride solution (P=7.5 atm), *Acinetobacter* formed a biofilm less intensively than *Pseudomonas*. When the osmotic pressure was increased to 27.4 atm, corresponding to a 3.0 % solution of sodium chloride, the intensity of film formation of bacteria of both species increased. The relative growth rate of optical density of biofilms, formed by Pseudomonas, reached about 20.0%, in comparison with optical density of biofilms, produced in conditions similar to blood plasma. In *Acinetobacter* the same criteria increased no more than 7.0%. Further increase in osmotic pressure in the environment has differently influenced the film-forming activity of *Acinetobacter* and *Pseudomonas*.

There was found that, in 6.0 % sodium chloride solution (P=52.14 atm), the intensity of film formation in *Acinetobacter* continued to increase, the optical density of the biofilms increased more than 30.0%, compared to films formed under isotonic conditions. Under these conditions the ability to biofilm production in *P.aeruginosa* was suppressed, since the optical density of biofilms dropped to values less than in isotonic conditions. There was proved, that a further increase in osmotic pressure in the culture medium (P=79.66; 9.0% sodium chloride solution) stimulated the activity of *A.baumannii* biofilm formation and further suppressed the film formation process in *Pseudomonas*.

It well known, that for treatment of patients with inflammatory processes, caused by *P. aeruginosa* and *A. baumannii*, such antibiotics as fluoroquinolones, aminoglycosides or carbapenems are often used. Determination of the biofilm forming activity in studied strains of the non-fermenting bacteria under the presence of levofloxacin, amikacin or meropenem in the nutrient medium demonstrated that meropenem and amikacin at sub-bacteriostatic concentrations did not significantly alter the intensity of their biofilm formation ($p>0.05$). In the presence of sub-bacteriostatic concentrations of levofloxacin, the activity of biofilm forming bacteria of both species increased significantly. The optical density of biofilms in the presence of the antibiotic was on average 25.0% higher, in comparison with the control (cultivation of bacteria in a medium without antibiotic).

In our research there were investigated the patterns of formation of resistance to levofloxacin, amikacin, mero-

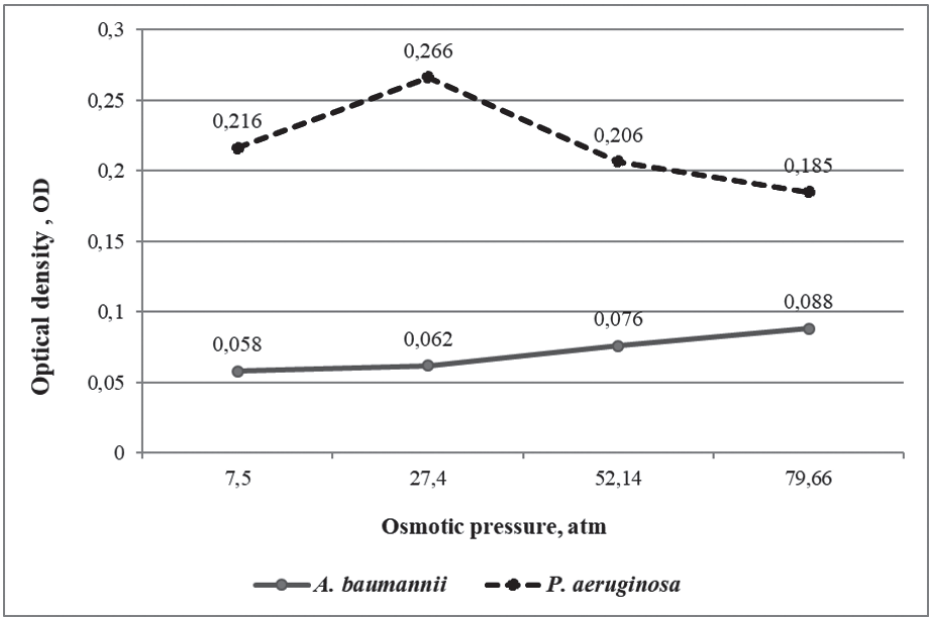

Fig. 2. The intensity biofilm forming activity of *P. aeruginosa* and *A. baumannii* in solutions with different osmotic pressure.

penem during the cultivation of non-fermenting bacteria in a nutrient medium with increasing concentrations of antibiotics [16]. And the artificially induced formation of resistance in these types of bacteria occured fairly quickly. By the tenth passage, the minimum bactericidal concentration of the drugs for most of the tested strains increased 16-fold, and to the 40th passage – almost 1000 times.

To study the intensity of formation of biofilms there were used isolates of bacteria, which had undergone the cultivation in an environment with increasing concentrations of antibiotics, and acquired the ability to grow in the presence of at least 16000 μg/ml of each of antibiotics, was used in the research. The biofilm forming activity of the original bacterial strains (not adapted to antibiotics) and the same strains after artificial adaptation to a medium with a high concentration of antibiotics was compared. According to the received data we found, that no statistically significant changes in the intensity of biofilm formation by the microorganisms of the genus *Acinetobacter* and *Pseudomonas* occurred after the procedure for adaptation to antibiotics. However, in most strains, a tendency to a slight decrease in biofilm forming activity was observed in antibiotic-adapted strains when grown in a non-antibiotic medium. This tendency was particularly stable in strains of *Acinetobacter* artificially adapted to meropenem.

DISCUSSION

The ability to rapid fixation on solid surfaces and formation of biofilms is an essential property of the Gram-negative non-enzymatic bacteria of the genus *Acinetobacter* and *Pseudomonas*, which helps them to survive in poorly favorable conditions and to inhabit numerous ecological niches [15, 16, 18]. It is difficult to explain the established ability of *Acinetobacter* to produce biofilms rich in organic matter in the absence of any sources of energy and plastic material in sterile water for injection. This only confirms the availability of great adaptive capacity in bacteria of this kind. According to the level of biofilm forming activity, non-fermenting bacteria exhibit heterogeneity between strains: some strains are capable of forming biofilms even under "hungry" conditions; others require the presence of nutrients in the environment. The stimulation the process of biofilm formation may occur in the environment of such simple organic substrates as glucose and urea. The additional administration of individual amino acids to the culture medium in our experiments did not enhance the biofilm formation by *Acinetobacter* and *Pseudomonas*, whereas in the presence of whole animal proteins in the form of animal serum, this process was significantly enhanced.

Pseudomonas were proved to exhibit a higher level of film-forming activity compared to *Acinetobacter* in an environment with osmotic pressure isotonic blood plasma (0.9% NaCl solution). Increasing osmotic pressure in the environment within certain limits stimulated the biofilm forming activity of bacteria of both species. However, *Acinetobacter* and *Pseudomonas* have different activity of biofilm formation under hypertonic conditions. There was determined the decrease of biofilm forming activity of the Pseudomonas isolates in conditions of the medium, containing 6.0 % sodium chloride. And this ability of *Acinetobacter* continued to increase, even in 9.0% salt solution. This phenomenon, along with the ability to form biofilms in distilled water, may indicate a higher environmental plasticity of *Acinetobacter* compared to *Pseudomonas*.

The presence of antibiotics in the environment is considered to be an unfavorable factor for bacteria as it should stimulate the processes of biofilm formation. However, we did not observe a similar effect against antibiotics of aminoglycoside and carbapenem series in our study. Fluoroquinolone antimicrobial levofloxacin at subbacteriostatic concentrations activated the biofilm formation of most

studied *Pseudomonas* and *Acinetobacter* strains. There is understandable the reduction of the biofilm forming activity of *Acinetobacter*, which have long been adapting to meropenem, in a non-antiobiotic environment. Obviously, the elimination of «antibiotic pressing» reduces the activity of the protective mechanism, which is biofilm formation.

CONCLUSIONS

1. In Gram-negative non-fermenting bacteria of the genus of *Acinetobacter* and *Pseudomonas* the ability to form biofilms, is a developed protective response under such adverse environmental effects as insufficient nutrients, increased osmotic pressure and the presence of antibiotic-containing substances in the environment.
2. Sodium chloride at high concentrations (6.0% or more) is able to suppress the activity of biofilm forming by *Pseudomonas*, at the same time stimulate this process in *Acinetobacter*.

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ORIGINAL ARTICLE

SLEEP DISORDERS IN RELAPSING-REMITTING MULTIPLE SCLEROSIS PATIENTS

DOI:10.36740/WLek202102115

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ABSTRACT

The aim: Our study aimed at evaluating the relationships between sleep disorders (SD), cognitive impairment (CI), anxiety and depression in patients with relapsing-remitting multiple sclerosis (RRMS).

Materials and methods: One hundred and five patients with RRMS (80 females and 25 males) aged from 22 to 67 years (mean age: 41,8±10,7; EDSS:3,5±1,6; disease duration (DD): 10,3±8,5 years) were enrolled into the study. All participants completed questionnaires on sleep (the Pittsburgh Sleep Quality Index /PSQI), cognitive functions (The Montreal Cognitive Assessment /MoCA), anxiety (Hamilton Anxiety Rating Scale /HAM-A), depression (Beck Depression Inventory/ BDI).

Results: According to PSQI score the patients were divided into two groups: with (n=42) and without SD (n=63). The patients with SD were older (45,36±1,66 vs 39,41±1,27, p=0.005), had higher EDSS score (3,98±0,26 vs 3,14±0,19, p=0,008), BDI (13,79±1,14 vs 8,96±0,86, p=0,0009) and HAM-A (24,52±1,42 vs 16,56±0,99, p<0,0001) scales compared with patients without SD. The frequency of anxiety (p=0,0034) and depression (p=0,038) was significantly higher in RRMS patients with compared to those without SD. No significant difference was found in gender, DD and MoCA score. In patients with SD significant negative correlation between MoCA and BDI score (r = -0,42, p<0,005) was found. In the group of patients without SD significant negative correlation between MoCA and EDSS (r = -0,27, p=0,03), MoCA and BDI (r = -0,26, p=0,043),) MoCA and HAM-A (r = -0,25, p=0,041) score was detected.

Conclusions: Insomnia type SD in RRMS patients were associated with older age, higher EDSS score and presence of anxiety and depression.

KEY WORDS: Multiple sclerosis, sleep disorders, cognitive impairment, anxiety, depression

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INTRODUCTION

Multiple sclerosis (MS) is a chronic progressive inflammatory autoimmune neurodegenerative disease of central nervous system (CNS) and it is considered to be the most prevalent neurological disability of young adults, often leads to severe physical or cognitive incapacitation [1]. The severity of disability in MS patients is increased not only by motor disorders and other neurological deficit, but by the presence of cognitive impairment (CI), psycho-emotional disorders, like anxiety and/or depression, and sleep disorders (SD) [2]. The frequency of SD in the MS population ranges from 47 to 62%, with a higher prevalence in women [3]. SD in MS can be either secondary due to numerous psychological or physical symptoms or primary [4]. In either of the two cases, a bidirectional relationship exists between MS and SD. Many factors like sex hormones, genetic mechanisms, psychosocial factors, certain physical factors that disrupt sleep, such as pain or bladder dysfunction may contribute to SD differences in female and male patients. SD in women are predominantly associated with depression and anxiety, whereas in men those are associated with pain [5]. MS patients prevalently suffer from insomnia, which is characterized by difficulties of falling asleep, maintaining sleep and early awakenings, inability to sleep as much as desired, which are predominantly accompanied by asthenia, excessive daytime sleepiness and/or disruption of daily activities [6, 7]. In order to establish the diagnosis of SD the foresaid symptoms must be present at least 3 nights per week for minimum three consecutive months [8].

The disease-modifying drugs or symptomatic therapies in MS also contribute to the development of SD. It was reported that sleep disturbance is a common complaint of patients with RR-MS receiving high doses of intravenous methylprednisolone [9]. Intake of IFN-β by RR-MS patients affects sleep continually, can increase fatigue, depression and negatively influences on the quality of life in general. Since this drug causes restlessness during sleep and difficult awakenings, both at initiation stage and after a chronic use, proving that these effects are not adaptive. However, the following side effect is reportedly cancelled by symptomatic therapies [10, 11], which can improve modulation of cytokines level and/or restore circadian secretion of melatonin and its suppressed metabolism [12]. Administration of glatiramer acetate leads to frequent awakenings during the night and daytime drowsiness due to increasing of anxiety and irritability, the typical side effects of the drug [13]. On the contrary, treatment with natalizumab or cannabinoids generally improves quality of sleep [14, 15]. Penner I.K. et al. demonstrated that natalizumab

administration had positive influence on fatigue, daytime sleepiness, cognitive function, depression and a quality of life in general from baseline to a year later [14]. Meuth S.G. et al. state that cannabis-based extracts improve general sleep disturbances as well as spasticity- and pain-related SD, but sudden discontinuation of such therapy caused interrupted sleep in 16% patients [15].

The relationships between sleep quality and clinical manifestations of MS is contradicting and not yet fully understood.

Our study aimed at evaluating the relationships between sleep disorders, cognitive impairment, anxiety and depression in patients with RRMS.

THE AIM

Our study aimed at evaluating the relationships between sleep disorders (SD), cognitive impairment (CI), anxiety and depression in patients with relapsing-remitting multiple sclerosis (RRMS).

MATERIALS AND METHODS

The current study consisted of one hundred and five patients with RRMS (80 females and 25 males) aged from 22 to 67 years (mean age 41,8±10,7). The maximum EDSS score was 6,0 with the mean score 3,5±1,6. Among the participants the minimum disease duration (DD) was one year and the maximum was forty-seven years (mean DD 10,3±8,5 years). All patients were diagnosed RRMS according to McDonald's Criteria 2010 [16]. A medical history was obtained from all the participants. The examination consisted of a standard clinical evaluation, neurological examination, the application of neuropsychological questionnaires, laboratory tests (complete blood count, biochemical parameters, TSH), MRI of brain and spinal cord. All the participants were screened for education. Education was divided into two categories: higher secondary school and higher vocational training for 18+ years/ university. To evaluate level of disability in MS patients Kurtzke's Expanded Disability Status Scale (EDSS) was applied. Mild disability equals 1-3,5 points, moderate – 4-6 points and 6,5-8 stand for severe disability [17]. The instrument applied to screen for SD presence and evaluate the quality of sleep was the Pittsburgh Sleep Quality Index (PSQI). PSQI scale consists of 19 items and 7 components: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, use of sleep medication, daytime dysfunction. According the test results 0-15 points mean absence of SD, 16-25 points – mild SD, 26-35 points – moderate SD and 36-45 points – severe SD [18]. The Montreal Cognitive Assessment (MoCA) was applied for assessment of cognitive functions. MoCA is a screening instrument composed of eight sections including visuospatial/executive functions, naming, memory, attention/processing speed, language, abstraction, delayed recall (short-term memory), orientation and education. The scale score is interpreted as: 30-26 points – no CI; 25-18 points – mild CI; <18 points – severe CI [19]. To find the presence and measure the severity of perceived anxiety symptoms we used Hamilton Anxiety Rating Scale (HAM-A). The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). Each item is scored on a scale from 0 (not present) to 4 (severe), with a total score range of 0–56, where 0-13- absence of anxiety, 14-17 indicates mild severity, 18–24 – moderate severity and ≥ 25 – severe anxiety [20]. Presence of depression was assessed by the means of Beck Depression Inventory (BDI). BDI is a 21-item self-report measure that taps major depression symptoms according to diagnostic criteria listed in the Diagnostic and Statistical Manual for Mental Disorders. Each answer is scored on a scale value from 0 to 3. Mean score 0–9 stands for absence of depression, 10–18 indicates mild depression, 19–29 – moderate depression and 30–63 – severe depression [21].

Recruitment criteria were as follows: patients older than 18 years with RRMS in stage of remission, EDSS less than 6,5 points, with SD in form of insomnia, without intake of sleep-modifying medications, absence of nocturnal pelvic disorders, absence of infectious diseases.

Participants were excluded if they were younger than 18, had progressive forms of MS, stage of exacerbation of RRMS or severe disability (EDSS score 6,5 – 8 points), severe depression, pelvic disorders, pregnancy, as well as patients treated with corticosteroids and INF-β, which could alter the study's parameters.

All the subjects provided written informed consent and the study was approved by the Institutional Ethics Committee.

Student's t-test (t) was applied for evaluating credibility between mean quantitative positions of two samples. Proportions were compared using $\chi 2$. The Pearson's correlation coefficient (r) between different indicators was analyzed. A value of $p<0,05$ was considered statistically significant.

RESULTS

The main complaints of the patients with RRMS were the following: inability to fall asleep, midnight awakenings, sense of psycho-emotional tension, inability to relax, inability "to turn off their head", presence of disturbing thoughts, anxiety, intermittent sleep, morning and daytime weakness, mood swings, increased irritability, fatigue, lack of energy during usual daily activities, memory decrease, vocabulary difficulties, inability to concentrate, decreased occupational performance. Neurological examination revealed brain stem disorders, pyramidal signs, pathological reflexes, increased muscle tone, impaired coordination (intention tremor, ataxia, missing the mark), sensory disorders (in particular, Lhermitte's sign).

According to the results of the brain MRI, the majority of the patients had multifocal lesions in the white matter, periventricular, cortical, juxtacortical, infratentorial areas, as well as lesions in the cervical segments of spinal cord.

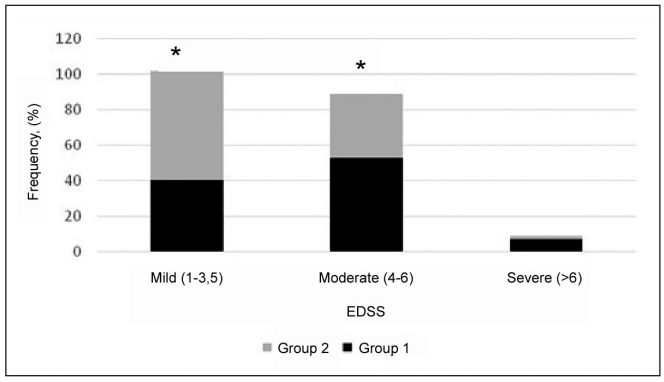

Fig.1. The frequency of different disability rate (according to EDSS) in the patients with RRMS of both groups
*-p= 0,005

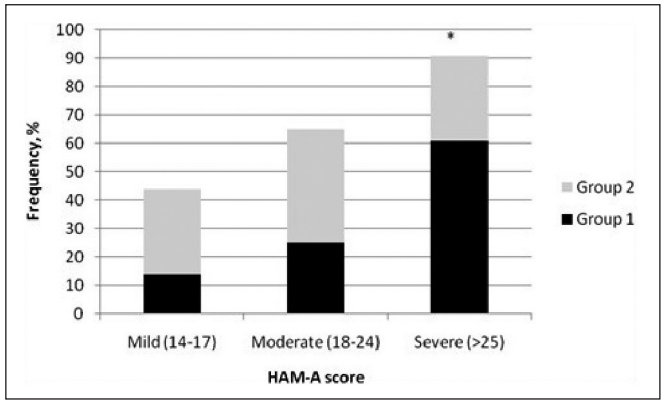

Fig.2. The frequency of various anxiety severity (according to HAM-A score) in the patients with RRMS of both groups
*-p<0,0001

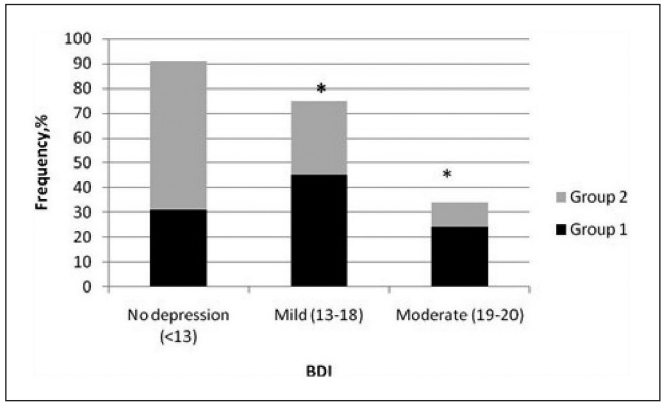

Fig. 3. The frequency of the various depression severity (according to BDI score) in the patients with MS of both groups
*-p=0,0001

According to PSQI the participants were divided into two groups: group 1 – with SD (n=42); group 2 – without SD (n=63). Analyzing the results of PSQI the most frequent indicators of SD were difficulties of falling asleep (25/60% patients), problems with maintaining sleep (23/55%), early awakenings (12/28%), restlessness and/or seizures of lower extremities (17/40%), daytime drowsiness (11/26%), lower daytime productivity (22/52%). None of the patients experienced nocturnal urinary problems, sleep-related breathing disorders (sleep apnea syndrome) or episodes of disorientation during the night. Based on this questionnaire, three levels of SD were observed: mild, moderate and severe. Mild SD were present in 18 (43%) patients, moderate – in 14 (33%) and severe SD – in 10 (24%) participants. 27 (64%) patients of the group 1 were older than 40 years, whereas in the group 2 this number was 31 (48%), so in proportion there is a tendency of developing SD coherent with aging, demonstrating a significant difference between groups according to the age of patients (p=0,005). Thus, the mean age of the group 1 (45,36±1,66) was substantially lower in comparison to the group 2 data (39,41±1,27). The group 1 consisted of 35 (83%) females, and 45 (71%) females were included in the group 2, which demonstrates no valuable difference.

We also considered the influence of higher education availability on presence of SD in MS patients. There were 17 (40%) patients with higher education in the group 1, as opposed to 39 (61%) people in the group 2. Thus, the frequency of patients with higher education was significantly lower in SD group of patients as comparing to those without SD (χ^2=8,82; p=0,003). The education level showed significant difference in both groups concerning its possible influence on sleep quality.

The group 1 demonstrated the following EDSS results: 17 (40%) patients had mild disability, 22 (53%) – moderate and 3 (7%) had severe level of disability; meanwhile 39 (62%) patients had mild disability, 23 (36%) – moderate and only one (2%) had severe disability in the group 2 (Fig1). The number of participants with mild level of disability was significantly lower meanwhile the moderate level of disability was much greater in the first group of patients with MS in comparison with patients of the second group (χ^2=10,7; p=0,005). The mean EDSS score was substantially higher (p=0,008) in SD group (3,98±0,26) comparing to those without SD (3,14±0,19).

The duration of disease did not differ significantly in both groups (11,62±1,35 in the group 1 and 9,44±1,03 in the group 2), hence its impact on development and severity of SD was irrelevant (p>0,05).

According to the MoCA score, 26 (62%) patients with SD had CI of different severity; meanwhile this indicator was 40 (63%) in patients without SD. The data of our study does not confirm the correlation between of SD and CI parameters (r=-0,25, p=0,10). No substantial difference was found in both groups since results were almost identical according to MoCA score (p=0,95).

In accordance with extended expertise, among the patients of the first group no CI was found in 16 (38%) individuals, 23 (55%) had mild and 3 (7%) had severe CI. Among those participants with mild CI the lowest parameters were found in such domains: visuospatial/executive functions (23/100%), memory (14/61%) and abstraction (12/52%); language (8/35%) and attention (7/30%) had relatively higher rates. All the patients with severe CI had poor performance in all cognitive domains with the lowest rate in memory and language.

The HAM-A scale results revealed the presence of anxiety in the patients with RRMS. There were 36 (86%) patients

suffering from anxiety in the group 1, among which 5 (14%) had mild, 9 (25%) – moderate and 22 (61%) had severe anxiety. Whereas, in the second group 37 (59%) had anxiety disorder, including 11 (30%) with mild, 15 (40%) – with moderate and 11 (30%) with severe level ($p<0,0001$) (Fig.2). Hence, the mean HAM-A score was 24,52±1,42 in the first group and 16,56±0,99 in the second ($p<0,0001$). Substantial positive correlation was found between HAM-A score and PSQI score ($r=0,47$, $p=0,003$), which indicates that presence of SD in patients with MS is strongly associated with anxiety level.

The assessment of BDI score revealed that in the group with SD 13 (31%) patients had no depression, 19 (45%) – mild depression and 10 (24%) – moderate one, severe level was not detected; whilst in the group without SD 38 (60%) patients had no signs of depression, 19 (30%) – mild, 6 (10%) – moderate, severe depression was not found as well (Fig. 3). There was significant difference concerning the frequency of depression's severity between the two groups of patients with MS ($p=0,0001$). The mean BDI score was 13,79±1,14 in the first group and 8,96±0,86 in the second ($p=0,0009$). Significant positive correlation between BDI and PSQI score was found ($r=0,37$, $p=0,047$), which indicates positive relationship between depression and SD, as well as the possible contribution of depression into the development of SD.

In both groups of patients with MS substantial negative correlation between MoCA and BDI score was detected (in the first group $r = -0,42$, $p<0,005$; in the second group $r=-0,26$, $p=0,043$ correspondently).

In the second group of patients the evidence of negative influence of disability, depression and anxiety on cognitive performance was found. According to the results of MoCA test, 23 (36%) participants (subgroup I) had unimpaired cognition, 30 (48%) (subgroup II) had mild CI and 10 (16%) (subgroup III) had severe CI. In accordance with EDSS score, 17 patients had mild disability and 6 had moderate in the subgroup I; 17 had mild, 13 – moderate disability in the subgroup II; in the subgroup III mild disability was in 6 patients and moderate – in 4 individuals. Thus, noteworthy correlation between MoCa and EDSS score was found ($r = -0,27$, $p=0,03$). As for HAM-A score, among patients without SD and CI (subgroup I) 11 participants had no anxiety, 3 patients – mild anxiety, 5 had moderate and 4 – severe; in the subgroup II 13 patients were without anxiety, 6 – with mild anxiety, 7 – with moderate and 4 had severe anxiety; in the subgroup III 4 patients had mild anxiety, 3 – moderate and 3 had severe level of anxiety. Noteworthy negative correlation between MoCA and HAM-A score was found in the patients without SD ($r = -0,25$, $p=0,041$).

DISCUSSION

Insomnia was prevalent in RRMS patients and associated with older age, that is congruent with the previous study [22]. Our findings show significant difference of education level concerning its possible influence on sleep quality in patients with RRMS. This result is consistent with data of Alhazzani A.A. et al. that the level of education is considered as a risk factor for development of insomnia on a condition of absence of depression [23]. We found no valuable difference between both groups concerning gender, that does not match with previously reported results that SD in MS patients are prevalently associated with female gender [9].

We failed to find any significant association between insomnia and duration of the disease in RRMS patients, that corresponds to data of other investigators *[22]. At the same time in our study patients with MS and SD had higher score on EDSS, that does not concur with findings of* Čarnická Z. et al [22].

In both groups of RRMS patients no significant difference was found according to MoCA score, unlike the results of other studies reporting the influence of sleep quality on cognitive function [24, 25]. Concurrent, all the RRMS patients with SD in the presence severe CI had poor performance in all cognitive domains with the lowest rate in memory and language. In patients with MS, in general, memory impairment was present in 40-60% cases already on early stages of the disease [26], information processing speed disorder was common in 12-25% of patients, executive functions were impaired in 19 % of MS [26, 27]. Thus, the more frequently affected domains were memory, attention, information processing speed and executive functions [28, 29]. Significant association between sleep disturbance and cognitive dysfunction was found in some studies [30-32]. In a systematic review by Hughes, A.J. et al., in particular, memory, executive functions were mostly affected in patients with SD [30]. Sleep disturbance was considered the predictor of future cognitive decline in MS; results of systemic review highlight the need to integrate sleep assessment into routine MS care. Interventions aimed treating sleep disturbance may offer promise for improving cognitive dysfunction in MS [30]. In patients with MS insomnia can additionally impact their vigilance, cognition, motivation and attention, greater amounts of sleep loss correlate with daytime sleepiness, poor cognitive performance and expressed fatigue [8, 33].

In our study presence of SD in RRMS patients is strongly associated with anxiety level. These findings are consistent with data provided by other investigators, which consider anxiety and stress as possible contributors to the development or progression of SD immensely due to brain mediators' malfunction [33]. We revealed positive correlation between depression and insomnia, that matches data of Bahmani D.S. et al., that emphasize the crucial effect of depression on the quality of sleep, and vice versa the deterioration of depressive disorders with presence of insomnia [32].

In both groups of RRMS patients substantial negative correlation between MoCA and BDI score was found. This finding is in accordance with previous studies, that state the influence of depression on the ability to learn, process information, impairs memory and practical skills. It predominantly occurs in case of location of lesions in temporal lobe, as the hippocampus and the amygdala, the structures

responsible for converting a short term-memory into a long term-memory, learning new skills and emotional reactions, can be damaged at the same time [34, 35]. Nocity V. et al. in their study revealed that poor sleep quality is associated with fatigue, higher scores of BDI and Self-Administered Anxiety Scale in MS patients [7].

All the mentioned above associations between SD, CI, anxiety and depression in MS patients can be explained by the fact, that sleep disruption affects CNS on the cellular level. Oligodendrocytes (OL) are the only cells of CNS with a function of myelination; hence any external or internal influence on those has crucial consequences. Maturation of OL depends on circadian cycle, disruption of which can be caused by SD or created artificially (common "disorder" in shift work). Neurodegenerative diseases associated with a disrupted sleep/wake cycle contribute to the volume of the white matter loss [25, 36, 38]. Philips T. and Rothstein J. D. reported that decreasing of sleep quality and duration has a negative influence on myelination due to OLs' dysfunction (disruption of myelin sheath and prevention of lactate transmission to axons) [24, 25].

In patients with RRMS without SD the evidence of negative influence of disability, depression and anxiety on cognitive performance was found. Our results are congruent with previous studies, according to which disability has crucial role in the development and deteriorating of CI in MS patients, but is often overlooked by physicians due to the vivid picture of neurological deficit [38]. Other authors connect MS with anxiety, since anxiety can be present independent of type, disease duration, level of disability or age and it deteriorates cognitive function and quality of sleep [39, 40].

The main strengths of our study are strict inclusion criteria and its use of a design adapted to evaluate sleep and psycho-emotional disorders, as well as the relationships between them, applying questionnaires. Although, several limitations need to be discussed. First, this study's cohort was only composed of RR-MS patients. Relapsing-remitting form is the most widespread form of MS. Therefore, our results cannot be generalized to SD prevalence in progressive forms of MS. Secondly, we had patients only with insomnia, restless leg syndrome and sleep apnea syndrome were absent in the study participants according to PSQI. Thirdly, SD were revealed only by applying the PSQI, not by other sleep quality questionnaires (Epworth Sleepiness Scale).

CONCLUSIONS

Insomnia type SD are prevalent in RRMS patients and are associated with older age, higher EDSS score and presence of anxiety and/or depression. Therefore, all MS patients with anxiety and/or depression should be screened for presence of SD since they tend to deteriorate patients' psycho-emotional condition and cognitive performance. The results of our study emphasize on the importance of interventions targeted at revealing sleep disorders and improving sleep quality in patients with MS for improvement of their non-motor symptoms and quality of life in general.

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ORIGINAL ARTICLE

CLINICAL FEATURES OF TOXIC JAW BONE OSTEOMYELITIS IN DRUG ADDICTS

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ABSTRACT

The aim: The aim of our research is to study the features of toxic osteomyelitis in drug addicts, their diagnosis and comprehensive treatment, aimed at strengthening motivation for the suspension of the use of psychoactive substances and the elimination of the pathological process in the lower jaw .

Materials and methods: The features of toxic osteomyelitis, complicated by abuse, have been studied on 46 patients in the department of oral and maxillofacial surgery of Lviv regional clinical hospital during 2013-2019. Psychoactive substances, used by the patients, varied from homemade drugs "Screw" taken by 32 men (69.5%), synthetic drug amphetamine consumed by 10 men (21,7%) to Subutex used by 4 patients (8,7%) for their pleasure. All the patients underwent comprehensive examination, which included clinical, laboratory, radiological, pathohistological studies. Almost all patients – 41 (89.1%) underwent sequestrectomy.

Results: Clinical picture progressed quite rapidly in the form of diffuse destructive-necrotic osteomyelitis of the mandible, which was characterized by a severe, atypical course of the pathological process with permanent inclusion of other additional areas of the lesion of the mandible. The X-ray at this stage showed an increase in destructive processes in the bone.

Conclusions: Appropriate surgical tactics and pathogenetic therapy are of great importance for toxic osteomyelitis in drug addicts. Due to the treatment, despite the total destruction of the mandible, it was possible to stop the destructive bone processes and to preserve life for such patients.

KEY WORDS: drug abuse, toxic osteomyelitis, Jaw bones

Wiad Lek. 2021;74(2):263-267

INTRODUCTION

A huge problem of modern medicine is a holistic approach to treating a patient, taking into account all the comorbid conditions resulting from an unhealthy lifestyle (abuse of psychoactive substances) and social disadvantage. At present, abuse has become one of the most serious global problems, caused by crisis of morality, culture, change of life values, and loss of sense of life, mostly affecting young people. All this, unfortunately, has led to sad statistics in Ukraine which holds the "palm of primacy" in Europe in terms of psychoactive substance use. According to official data alone (the Ministry of Health of Ukraine 2019 report on people with mental disorders due to the use of all groups of psychoactive substances) in Ukraine, more than 519 thousand persons are being monitored , with 452 thousand of them having mental disorders and behavior due to alcohol consumption, and 40-42 % of patients having comorbid pathology. One of the diseases, which often occurs during drug abuse, is toxic osteomyelitis of bones. The factual material, covered in considerable detail in literature sources [1, 2, 3, 4], enables us to detail the clinical picture of this serious disease. According to the authors V.O.Malanchuk and , I.S.Brodetsky (2009, 2010, 2013 , 2014) the total number of patients with drug abuse is 60% of the total amount of patients with osteomyelitis of the jaws without comorbid pathology. The authors studied etiology, pathogenesis, clinical pictures and the ways to overcome the disease. It was revealed that the patients have atypical occurrence of odontogenic processes against the background of drug addiction with serious long course, frequent relapses, rapid spread of the pathological process, low efficiency of traditional treatment regimens, which is directly connected with a lengthy use of drugs. The effects of a prolonged use of psychoactive substances are rejection, sequestration of parts of the jaws, loss of natural teeth [5,6,7]. The process of damaging the bone tissue of jaws sometimes lasts for months or years. At the same time the patient completely rejects the fact that it is the substance abuse that has such serious consequences. Only a small percentage of patients think about the cause , which has led to such a condition, and refuse from psychoactive substance abuse, which helps to stop the development of the pathological process in the jaw .

THE AIM

The aim of our research is to study the features of toxic osteomyelitis in drug addicts, their diagnosis and comprehensive treatment, aimed at strengthening motivation for the suspension of the use of psychoactive substances and the elimination of the pathological process in the lower jaw .

MATERIALS AND METHODS

To study the features of toxic osteomyelitis, complicated by abuse, 46 patients of the department of oral and maxillofacial surgery of the regional clinical hospital in Lviv were examined during the period from 2013 to 2019. The average age of the patients (men) was 26.5 ± 6.57. In all patients, the pathological process was localized in the mandibular area. Twenty-nine patients (63.0%) people, the majority of patients, took drugs for more than 5 years; 15 (32.6%) patients took drugs for not more than two years. The patients mainly consumed hard drugs or homemade drugs for up to two years, which is quite harmful for health .

The clinical picture of toxic osteomyelitis in psychoactive substance addicts directly depended on the duration of abuse and the composition of drugs. In 10 (21.7%) patients with long-term use of psychoactive substances > 5 years , the pathological process progressed rapidly due to dense and thick cortical plates, which hindered the break of pus on the outer surface of the bone, with high intoxication and damage to new parts of the jaw .

Having given their consent, all patients underwent comprehensive examination, which included clinical, laboratory, radiological, pathohistological studies. When collecting the history from patients, it was found that all of them consumed drugs. All this was very important for further treatment , because the patients tried to conceal that they took drugs and were addicts. Psychoactive substances, used by the patients, varied from homemade drugs "Vint" taken by 32 men (69.5%), synthetic drug amphetamine consumed by 10 men (21,7%) to Subutex used by 4 patients (8,7%) for their pleasure .

All patients underwent complex treatment with surgical intervention and subsequent medication, with establishing contact between doctor and patient . The effectiveness of the treatment depended on many factors such as age of patients, the duration psychoactive substance abuse, the condition of the patient upon arrival and others.

Almost all patients - 41 (89.1%) underwent sequestrectomy, after which the prescription of antibiotics was not the only drug treatment, since patients with osteonecrosis of the jaws with drug addiction have deep pathological changes in various systems of the body (immune, cardiovascular, hematopoietic, hemostatic). All other patients did not require surgery, since the destruction of bone tissue (according to the results of CT) was not accompanied by the formation of sequesters, or their sizes were insignificant, and they came out through the fistula.

Moreover, in the patients with pathogenic addiction, the microflora is highly resistant to antibiotics. Therefore, the combined use of systematic enzymotherapy (namely wobenzym) and intramuscular administration of clindamycinum resulted in a high concentration of the antibiotic in pyo-necrotic focus of the bone tissue. Clindamycinum has an immune modulating property and is a derivative of lincosamides, effectively used to treat osteomyelitis. We have proposed the introduction of 5 ml clindamycinum intramuscularly 2 times a day for 10 days. Clindamycin should be used with caution in patients with severe hepatic impairment. In our research group, severe liver pathology was not observed in either instrumental or clinical studies.

With severe pain, 32 (69.6%) of 46 patients were offered nimesulide, included in the group of non - steroidal anti - inflammatory drugs, 1 tab. 3 times a day to reduce pain within 5 days. All 46 patients were prescribed wobenzym, 1 tab. 3 times a day. It consists of highly active enzymes of plant and animal origin and has anti-inflammatory, anti-edematous effect and fibrinolytic effect, which allowed to normalize the immune imbalance and to increase the therapeutic effectiveness of antibiotics. The therapeutic effect of the drug is aimed at accelerating the lysis of toxic products, which contributed to the improvement of microcirculation .

Given the complex comorbid pathology, the integrated approach to medical treatment was extremely relevant and was directed at improving vital parameters by using the drugs that eliminate, first of all, immune imbalance, and enhance the overall condition of the body. At the beginning of treatment, for detoxification, all patients were prescribed 15 mg enterosgel 3 times a day for 14 days. The narcologist, in his turn, prescribed 24 mg polioksidony three times a day. This drug has a clearly pronounced immuno modulating action, detoxification and antioxidant activity, removes toxins and salts of heavy metals from the body, helps to inhibit peroxide oxidation of lipids, which provides an increasing resistance of the organism to the local and generalized infections. Twenty-nine (63.0%) patients, who were in a state of withdrawal syndrome remission, were prescribed hidazepam 50 mg 3 times a day sublingually. After taking the drug that has a mild tranquilizing and anxiolytic effect with antidepressant components,

psychomotor agitation, anxiety and irritability almost did not appear.

For a better understanding of the problem of the features of toxic osteomyelitis in addicts, we fully described the entire clinical picture of comorbid pathology and its treatment on the example of one of our patients who came to the clinic after the occurrence of edema of admaxillary soft tissue edema. The patient at once confirmed the long-term use of psychoactive substances (4 , 5 years), which allowed avoiding relapse in the postoperative period, using an integrated approach to treatment.

RESULTS

Most of the patients who participated in our study used hard or artisanal drugs for at least two years. Of the whole group, 4 participants (8.7%) used Subutex. When collecting the anamnesis, it was found that these patients used subutex as a drug intravenously for four years. The condition of these patients in terms of the severity of the concomitant pathology, toxic osteomyelitis, did not differ from the patients who abused other drugs, which did not contradict our study.

The toxicity of various impurities of homemade drugs (such as free iodine) lead to necrotic-degenerative processes in bone tissue, which is the cause of osteomyelitis and total bone destruction.

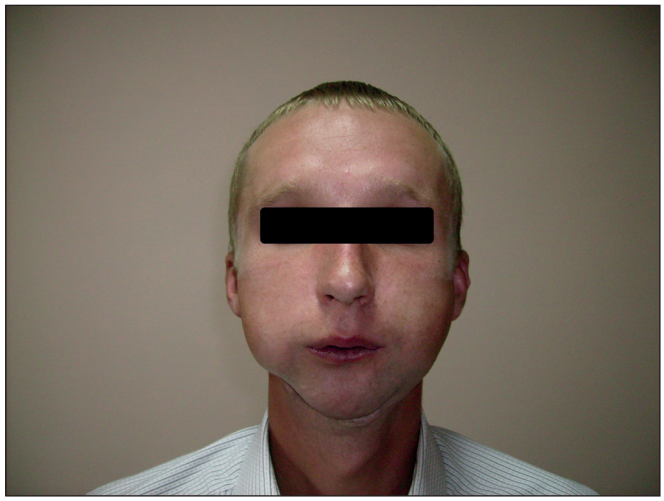

Fig 1. Photograph of the patient in the frontal projection at the end of diffuse osteomyelitis in the lower jaw: there is a decline of soft facial tissues in its lower third

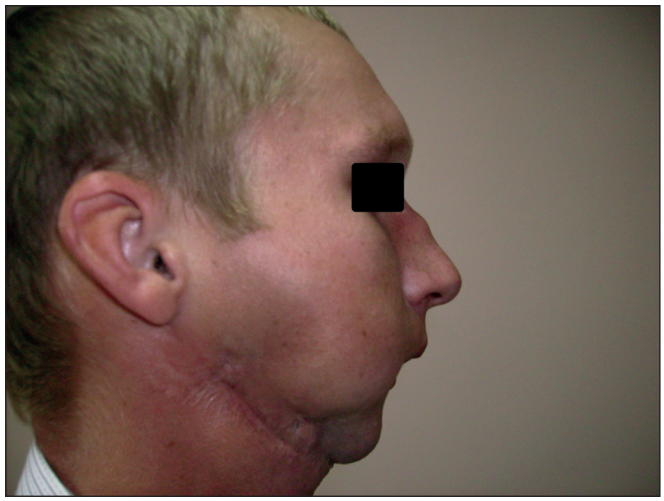

Fig 2. Photograph of the same patient in lateral projection: in addition to soft tissue sinking, in the upper cervical area, there are scars from multiple surgical interventions.

During the first clinical examination, an asymmetric face was observed in the patient due to swelling and infiltration of the soft tissues. These phenomena were known to have occurred after exacerbation of chronic periodontitis of 46 tooth and its removal. The patient indicated that he has many years of experience in the use of psychotropic substances of the primary series.

In the following days, with the spread of the inflammatory process, the patient felt pain on palpation of the relevant parts of the bone, and a significant thickening of the jaw was visually observed. Prolonged intoxication provoked aching pains, which were associated with the appearance of new foci of osteomyelitis and relapses, as well as significant fistulas.

Further, the clinical picture progressed quite rapidly in the form of diffuse destructive-necrotic osteomyelitis of the mandible, which was characterized by a severe, atypical course of the pathological process with permanent inclusion of other additional areas of the lesion of the mandible. Several times after the formation of bone sequesters, the patient underwent sequestrectomy surgery and was prescribed appropriate treatment, which included not only a combination of surgical, medication and orthopedic methods, but also consultation of a physician-narcologist and physician-psychotherapist for further treatment of addiction. (Figs. 1, 2).

The X-ray at this stage showed an increase in destructive processes in the bone. The final orthopantomogram showed total destruction and complete absence of the chin, body and angles of the mandible and, partially, up to ½ the height of the branches of the mandible on both sides. On the cult of the mandibular branch to the left there is a radiological shadow of the reconstructive plate, which was used as an immobilizing agent of the jaw fragments, which were further lysed (Fig. 3).

DISCUSSION

Research into literature sources indicated that by the conscious domestic use of psychotropic substances, Ukraine is one of the countries where it makes up a significant percentage, and the problem itself is becoming more and more social, especially among the younger generation [1, 2].

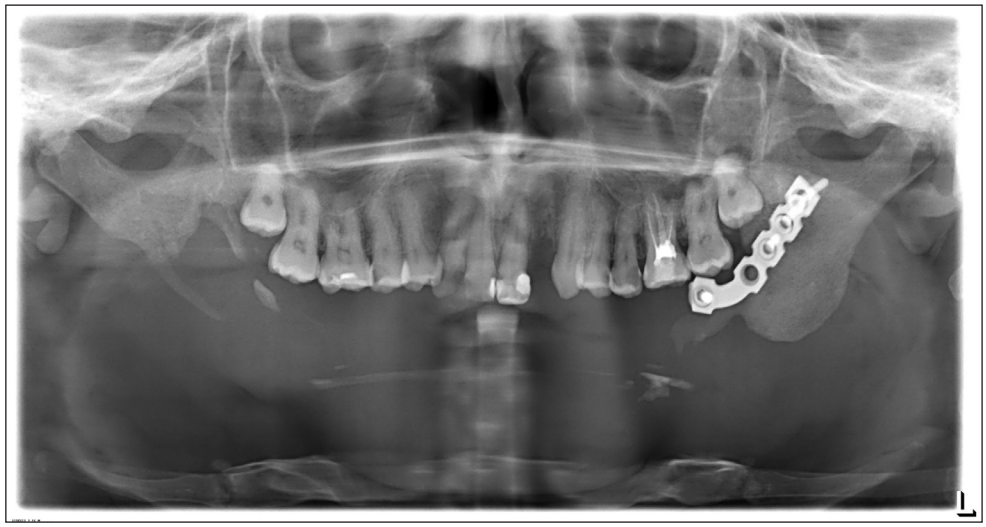

Fig 3. Orthopantogram of a patient with many years of experience in the use of narcotic drugs of the primary series.

Our observations, which completely coincide with other scientific studies, reveal that up to 40-42% of such adherents also have various comorbid pathologies, which significantly complicates their treatment and social adaptation [3, 4].

Working in the Department of Maxillofacial Surgery of LNMU, we have recently found a significant increase in diffuse toxic osteomyelitis of jaw bones, which is also confirmed by the data of the studied scientific works, and their percentage reaches 60% [2, 7]. Analysis of case histories of such patients showed an atypical course of odontogenic processes against the background of drug dependence with a severe long-term course, frequent relapses, rapid spread of the pathological process, low efficiency of traditional treatment regimens, which is directly related to long-term drug use. The consequences of long-term use of psychoactive substances are rejection, sequestration of jaw parts, loss of natural teeth [5,6,7]. The process of damage to the jaw bone tissue sometimes lasts for months or years. At the same time, the patient completely rejects the fact that the very abuse of psychoactive substances has such serious consequences [3].

Conducting this study, the authors set the task to study the peculiarities of toxic osteomyelitis in drug addicts, their diagnosticss and comprehensive treatment, which aims to increase motivation to suspend the use of psychoactive substances and eliminate the pathological process in the lower jaw.

To study the peculiarities of toxic osteomyelitis, complicated by drug abuse, 46 patients of the Department of Maxillofacial Surgery of the Lviv Regional Clinical Hospital were examined during the period from 2013 to 2019. The average age of patients (men) was 26.5 ± 6.57. In all patients, the pathological process was localized in the lower jaw. Twenty-nine (63.0%) persons, who made up the majority of patients, had been on drugs for more than five years; 15 (32.6%) people had taken drugs for no more than two years.

The clinical picture of toxic osteomyelitis in people who abuse psychoactive substances directly depended on the duration of use and their composition. Thus, in 10 (21.7%) patients with long-term use of psychoactive substances> 5 years, the pathological process developed rapidly due to dense and thick cortical plates, which made it difficult for pus to break through to the outer surface of bone, with high intoxication and damage to new parts of jaws.

All patients underwent comprehensive treatment with surgery and subsequent medical treatment with the contact established between doctor and patient. Almost all patients – 41 (89.1%) persons underwent sequestration, after which, given that patients with jaw osteonecrosis against the background of drug dependence have profound pathological changes in various body systems (immune, vascular, hematopoietic, hemostasis), medical treatment was not limited to the prescription of antibiotics. Moreover, in drug dependent patients, pathogenic microflora has high antibiotic resistance.

Therefore, systemic enzyme therapy (namely, wobenzyme) and administration of clindamycin, used simulnaneously, provided a higher concentration of antibiotic in the purulent-necrotic focus of bone tissue. We proposed the intramuscular administration of Clindamycin 2 times a day for 5 ml for 10 days. In case of severe pain, 32 (69.6%) patients out of 46 were offered Nimesulide, which belongs to the group of nonsteroidal anti-inflammatory drugs, 1 tab. 3 times a day for 5 days to reduce pain. All 46 patients were prescribed Wobenzyme,1 tab. 3 times a day, which includes highly active enzymes of plant and animal origin with anti-inflammatory, anti-edematous, fibrinolytic effect, which made it possible to normalize the immune imbalance, increase the therapeutic efficacy of antibiotics. The therapeutic effect of the drug is aimed at accelerating the lysis of toxic metabolic products, which improved microcirculation.

Given the complex comorbidity, a comprehensive approach to drug treatment was essential and aimed at improving vital signs with the use of drugs that eliminate, first of all, immune imbalance, and strengthen the general condition of the body. At the beginning of treatment, for detoxification, all patients were prescribed Enterosgel, 15 mg 3 times per day for 14 days.

The narcologist, in turn, offered the patients 24 mg of Polyoxidonium 3 times per day. The drug has a pronounced immunomodulatory effect, detoxifying and antioxidant activity, removes toxins, salts of heavy metals, and inhibits lipid peroxidation, which increases the body's resistance to local and generalized infection. Twenty-nine (63.0%) patients who were in remission of the withdrawal syndrome were prescribed Gidazepam, 50 mg 3 times a day, sublingually. After taking the drug, which has a mild tranquilizing and anxiolytic effect with antidepressant components, psychomotor agitation, anxiety and irritability were almost non-existent.

We believe that the obtained positive results allow us to consider this set of measures as the one that reduces the percentage of disability of patients with a stable refusal to abuse psychoactive substances and allows saving the life of such patients and create conditions for subsequent reconstructive surgery.

CONCLUSIONS

Appropriate surgical tactics and pathogenetic therapy are of great importance for toxic osteomyelitis in drug addicts. Therefore, in our study, with long-term integrated treatment, systemic and pathogenetically conditioned drug therapy was used, and appropriate surgical treatment, aimed at removal of necrotized areas of the mandibular bone, was performed. We believe that due to the treatment, despite the total destruction of the mandible, it was possible to stop the destructive bone processes and to preserve life for such a patient and to create conditions for subsequent reconstructive operations. The treatment was aimed at reducing the disability of patients with the persistent rejection of substance abuse.

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ORIGINAL ARTICLE

BIOLOGICAL METHOD FOR BABESIOSIS DETECTION: THE UNIFIED VERSION IN VIVO

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ABSTRACT

The aim is to establish a unified version of the biological method for babesiosis detection in vivo.

Materials and methods: samples (n=257) of biological material of different origin were examined. These included: blood samples from patients (n=6) and cattle (n=15); salivary gland homogenates (n=28) from 147 imagoes of ticks of the family Ixodidae, 32 imagoes of Ixodes ricinus and 115 imagoes of Dermacentor reticulates; spleen homogenates (n=63) from mouse-like rodents (Muridae) of the genera Myodes, Microtus, Apodemus and Sylvaemus. In order to cultivate in vivo Babesiae of the species B. microti, Syrian hamsters were infected with spleen homogenates from mouse-like rodents; for cultivating the B. divergens species Mongolian gerbils and nonlinear white mices were infected with blood samples from patients and cattle and salivary gland homogenates from ixodic ticks. The technology of modeling was based on the group specificity (differences in susceptibility to parasites and in parameters of morbidity) of the animals, involved in the experiment (Syrian hamsters, Mongolian gerbils, nonlinear white mices).

Results: Experimental animals were contaminated by means of intraperitoneal inoculation of 0.3 ml samples of biological material (infected with Babesiae). The animals were infected next day following a day of their preinoculation preparation. The marker parameters for the functional state of experimental animals were as follows: preterm death; appearance and development of clinical-laboratory signs of disease (hypo- or adynamia, loss of appetite, inertness/absence of reactogenicity to tactile/acoustical stimulation, postural changes, wetting of fur, pronounced lameness, flatulence, loss of ≥ 25% of body mass) in them; parasitaemia, histodestruction, cellular detritis. Parasitaemia was detected every two days (beginning with day 8 from the moment of inoculation) by reserves of light and luminescent microscopy. In case of the positive result (revealing of haemoparasites with Babesia spp.-like morphological and tinctorial signs) the verification of Babesiae with their more precise specific identification was performed using the technique of polymerase chain reaction (PCR). Preliminary detection of morbidity parameters in each experimental animal with the artificially created immunocompromised state became an obligatory moment of the described experiment.

Conclusions: The biological method for detecting Babesia spp. in vivo was improved by the author. This result was achieved by using a double reservoir (Syrian hamsters, Mongolian gerbils and nonlinear white mices with an increased level of susceptibility to parasites) followed by the immunocompromise formation. The use of the improved version of biological method increased the total rate of revealing of Babesiae, therewith creating an objective basis for optimizing the available ways of detection and study of Babesiae in vivo.

KEY WORDS: biological method, detection, Syrian hamsters, Mongolian gerbils, nonlinear white mices, babesiosis

Wiad Lek. 2021;74(2):268-272

INTRODUCTION

Babesiosis as a little-studied emergent haemoparasitic infection presents certain difficulties in its detection and differential diagnosis [1-3]. Species specificity of the causative agents of the above disease determines the conjuncture of both etiopathogenetic scenario of babesiosis and its numerous clinical episodes, the catamnetic plot [4-6]. A full understanding of the latter demands that researchers (including in the sense of creating a rich diagnostic evidential base) both use modern novel ways and orient themselves on (apply) a time-tested classic of the genre (in which experimental modeling takes not the last place). An expected contribution to solving the above problem is made by combined diagnostics. Hence, designing and development of improved clinical-diagnostic algorithms of babesiosis are an urgent problem of both theoretical and fundamental fields of medicine.

THE AIM

The aim is to establish a unified version of the biological method for babesiosis detection in vivo.

MATERIALS AND METHODS

In order to reveal and cultivate the causative agents of babesiosis (Babesia spp.) we studied samples (n=257) of biological material of different origin. These included: blood samples from patients, who lived in the Kharkiv Region, with diagnosed Lyme disease resulting from a tick bite (n=6); cattle (n=15) with suspected Babesiae invasion (the Chernihiv and Poltava Regions, Ukraine). The sample scope also included salivary gland homogenates (n=28) from 147 imagoes of ticks of the family Ixodidae, 32 imagoes of Ixodes ricinus and 115 imagoes of Dermacentor reticulates, taken from resources of field studies

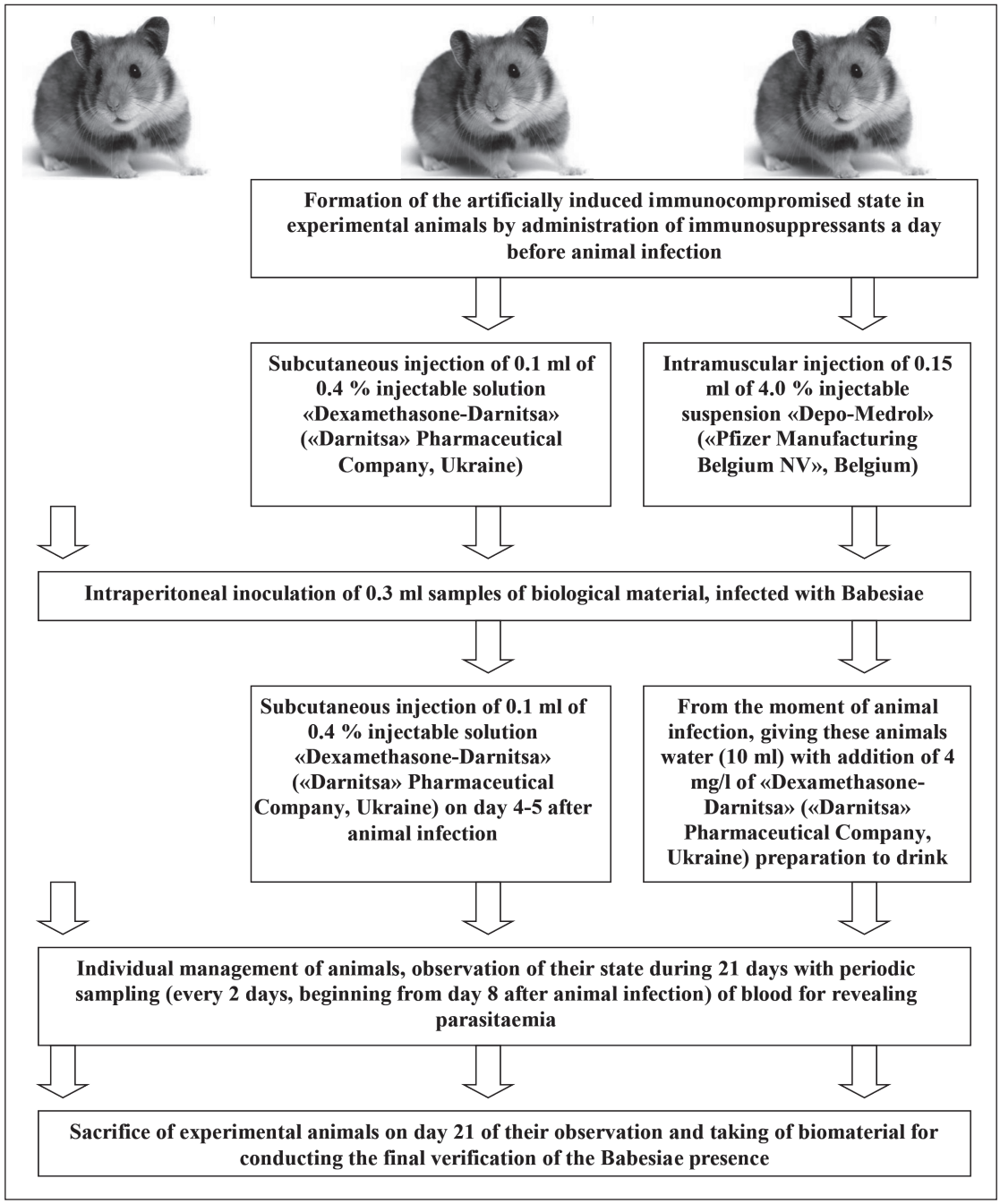

Fig. 1. The algorithm of detection and cultivation of Babesiae in vivo with use of Syrian hamsters.

in epizootic areas of the Kharkiv, Volyn, Chernihiv and Zhytomyr Regions (Ukraine). Spleen homogenates (n=63) from mouse-like rodents (Muridae) of the genera Myodes, Microtus, Apodemus and Sylvaemus, caught in natural ecotopes of the Zhytomyr, Poltava and Kharkiv Regions (Ukraine), were an obligatory object of the described study.

Detection and in vivo cultivation of Babesiae (the species B. microti) were made on males and females of Syrian hamsters (Mesocricetus auratus, n=45) with body mass of 30-45 g and at the age of 9-22 weeks [7-11]. Proper experimental procedures with the species B. divergens were performed on males and females of Mongolian gerbils (Meriones unguiculatus, n=55) with body mass of 45-90 g and at the age of 9-22 weeks and nonlinear white mices (n=45) with body mass of 11-18 g and at the age of 4-6 weeks [6-11].

The laboratory animals with proper documents, presented in the appropriate registration form, from the veterinary service of Ukraine were placed under quarantine and managed in standard conditions («climate-control») at the vivarium of the State Institution «Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine».

Air temperature was 18-24°C, taking into account the poikilothermy of each animal; relative humidity did not exceed the level of 50-70%; artificial illumination was normally maintained with the value of 60 lx with a 12-

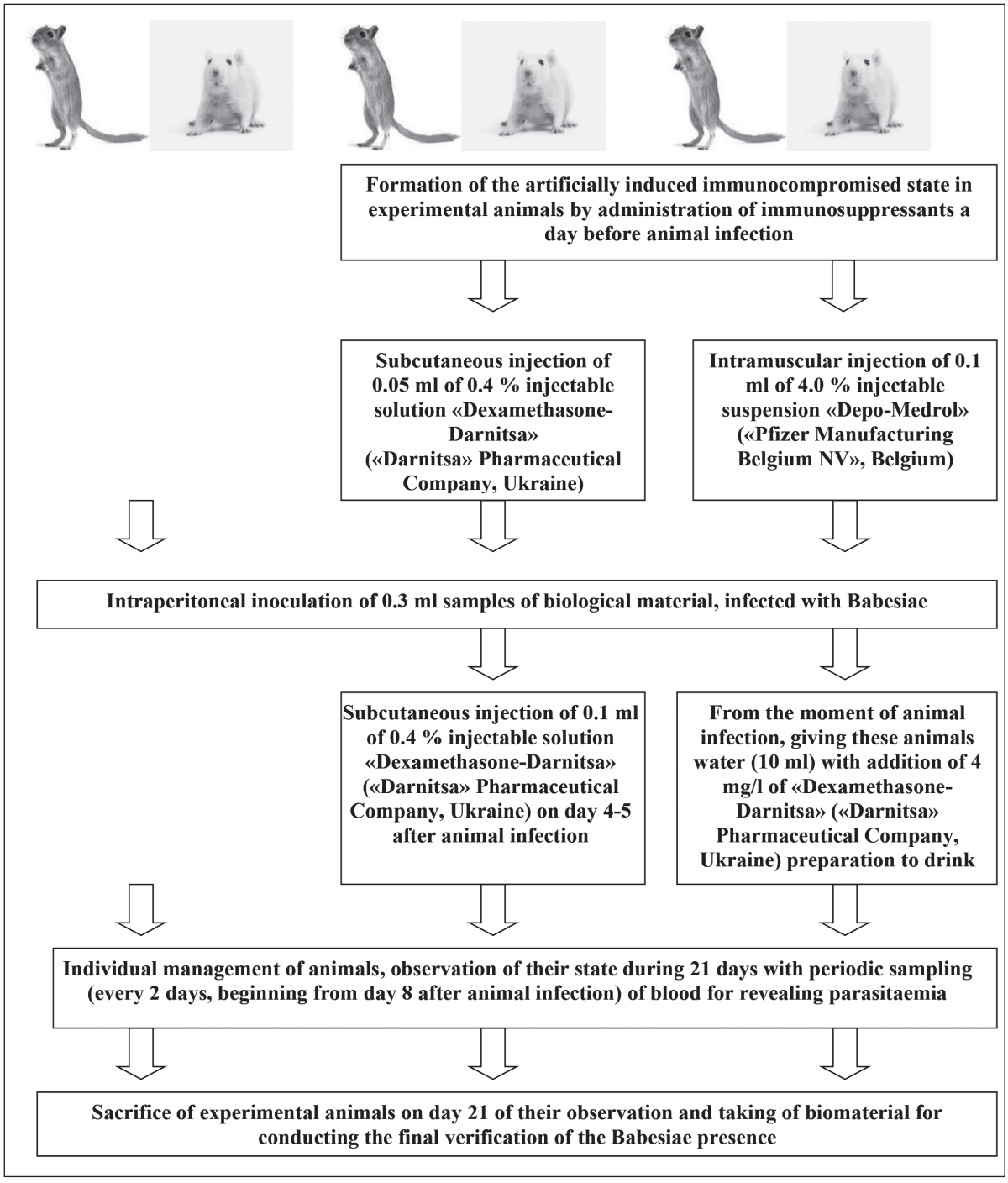

Fig. 2. The algorithm of detection and cultivation of Babesiae in vivo with use of Mongolian gerbils and nonlinear white mices.

hour cycle. Manipulations with selection, preparation and studying of biological material samples were performed with observation of rules of aseptics for the maximally possible prevention of their additional contamination with nonstudy microflora [4, 5] at operating rooms of the vivarium of the State Institution «Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine» with a direct participation of a veterinary doctor in morning hours. The latter fact was caused by biorhythmical activity and chronobiological features of laboratory animals [1]. From the morning of the day of the experimental intervention any feeding of the animals was excluded. During the whole nonexperimental period the feeding of the animals was organized in strict compliance with developed scientific standards, was of the rational and balanced nature, and conformed with daily requirements of feeding stuffs (with calculations considering weight, age and sex indices of each animal). Access to water and food remained free. Standard extruded foodstuff and water were given in sufficient quantity (in the amount of 30-32 g per each animal in order to avoid cases of cannibalism) two times a day (in the postexperimental period ad libitum) with periodic analysis of foodstuff samples for microbiological contamination [1]. Taking into

consideration that the rodents, used in the experiment, were nocturnal animals and ate in hours of darkness too, most of their foodstuff was given in the evening, at 8 p.m. [3]. All animals were examined every day by a veterinary doctor, who exercised control over the animals' vital activity during the whole experiment (postprocedure/ manipulation period, sampling of biological material, removal from the experiment) [7]. Sacrifice was made by hyperanaesthetization with chloroform. The conditions of animal management and care satisfied requirements of international and national documents.

RESULTS AND DISCUSSION

The biological method of babesiosis diagnosing in humans is to detect Babesia spp. by growing them in vivo in the body of sensitive experimental animals [1-4].

In order to cultivate in vivo Babesiae of the species B. microti, Syrian hamsters were infected with spleen homogenates from mouse-like rodents (fig. 1). In order to detect and cultivate in vivo Babesiae of the species B. divergens, Mongolian gerbils and nonlinear white mices were infected with blood samples from patients with Lyme disease, from cattle with Babesiae invasion and with salivary gland homogenates from ixodic ticks (fig. 2).

In order to objectify elements of reproduction of biological method, detection and cultivation of Babesia spp. we carried out preinoculation preparation of laboratory animals for formation of the induced (acquired) immunocompromised state in them. The latter facilitated in improving susceptibility of experimental animals to parasites and provided conditions for intensive growth of the above parasites in the infected organism and formation of morbidity.

The technology of modeling was based on the group (n=3) specificity (differences in susceptibility to parasites and in parameters of morbidity) of the animals, involved in the experiment (Syrian hamsters, Mongolian gerbils, nonlinear white mices).

The control group (G 1) consisted of 103 animals (31 Syrian hamsters, 41 Mongolian gerbils, 31 nonlinear white mices), who were infected with biomaterial samples without any preinoculation preparation. The immunocompromised state in the above animals was absent.

The second group (G 2) was made up 21 animals (by 7 animals in each subgroup of Syrian hamsters, Mongolian gerbils, nonlinear white mices). The above animals were characterized by an artificially created immunocompromised state. The latter was formed by subcutaneous injections of 0.05 ml (for Mongolian gerbils and nonlinear white mices) or 0.1 ml (for Syrian hamsters) of 0.4 % injectable solution «Dexamethasone-Darnitsa» («Darnitsa» Pharmaceutical Company, Ukraine). The experimental animals were contaminated by means of intraperitoneal inoculations of 0.3 ml samples of biological material (infected with Babesiae). The experimental specificity of the test animals from G 2 consisted in the fact that on days 4-5 after their contamination with biomaterial samples they were given another injection of 0.4% injectable solution «Dexamethasone-Darnitsa» («Darnitsa» Pharmaceutical Company, Ukraine).

The third group (G 3) included 21 animals (by 7 animals in each subgroup of Syrian hamsters, Mongolian gerbils, nonlinear white mices). The artificially induced immunocompromised state was achieved by intramuscular injections of 0.1 ml (for Mongolian gerbils and nonlinear white mices) or 0.15 ml (for Syrian hamsters) of 4.0 % injectable suspension «Depo-Medrol» («Pfizer Manufacturing Belgium NV», Belgium). After that, the animals were infected according to the developed scheme. Peculiar for the above experimental group were manipulations with giving these infected animals water (10 ml) with addition of 4 mg/l of «Dexamethasone-Darnitsa» («Darnitsa» Pharmaceutical Company, Ukraine) preparation to drink.

Preliminary detection of morbidity parameters of each animal with the artificially formed immunocompromised state was an obligatory component of the described experiment. The last manipulation was targeted at revealing a potential presence of the causative agents (wild strains) in the organism of experimental animals involved in the study. By the results of our observations (21 days) the whole sample scope of noninfected imunnocompromised animals went out of the test experiment without any cases of lethality, disease and asymptomatic parasitaemia. It is this contingent of animals that was engaged in further experiments with biological method (in vivo) for detecting and cultivating Babesia spp.

The marker parameters for the functional state of experimental animals of G 2 and G 3 were as follows: preterm death; formation and development of clinical-laboratory signs of disease (hypo- or adynamia, loss of appetite, inertness/absence of reactogenicity to tactile/acoustical stimulation, postural changes, wetting of fur, pronounced lameness, flatulence, loss of ≥ 25% of body mass); parasitaemia, histodestruction (marked inflammatory reactions, destruction of title cells of marker organs, necrosis), large accumulation of cellular detritis. Detection of parasitaemia (search for intra- and extraerythrocytic inclusions) was performed every two days (beginning from the moment of inoculation) by reserves of light and luminescent microscopy. In case of the positive result (revealing of haemoparasites with Babesia spp.-like morphological and tinctorial signs) the verification of Babesiae with their more precise specific identification was performed using the technique of PCR. The latter was carried out in its standard format using commercial test systems (manufactured by «IsoGene Lab. Ltd», the Russian Federation): the kit of reagents for amplifying deoxyribonucleic acid (DNA) of Babesia spp. «Gene Pak®PCR test» (with a system of Bab primers), Cat. No. E 2130, the kit of reagents for amplifying DNA of Babesia microti «Gene Pak® PCR test» (with a system of Bmi primers), Cat. No. B 2129 and the kit of reagents for amplifying DNA of Babesia divergens «Gene Pak®PCR test» (with a system of Bdi primers), Cat. No. E 2126, respectively.

The animals, which died before their time or were sacrificed (the final terms of the experiment), underwent an

obligatory autopsy. The latter was performed in conditions of a veterinary dissection room with a thorough somato-organoscopic analysis, detailed revision of abdominal organs, revealing of topographic specificity, holo-, skeleto-, syn- and vasotopy. The above was followed by taking of the organs, targeted for babesiosis (spleen, liver, stomach and small intestine; as for the last two, with consideration of the response of their lymphoid structures). Blood was received by the puncture method of heart or lingual artery (while the animals were alive). Blood samples were used for making model test specimens in the context of immunological studies of consequences of Babesiae invasion for detecting dominant species. Fragments of targeted organs were kept in RPMI 1640 (Sigma-Aldrich, USA) medium at t^o= -32^{o}C for receiving cultures.

CONCLUSIONS

The biological method for detecting Babesia spp. in vivo was improved by the author. This result was achieved by using a double reservoir (Syrian hamsters, Mongolian gerbils and nonlinear white mices with an increased level of susceptibility to parasites) followed by the immunocompromise formation. The use of the improved version of biological method increased the total rate of revealing of Babesiae (up to 35.7%), therewith creating an objective basis for optimizing available ways of detection, cultivation and further in vivo investigation of Babesiae as a group of little-studied causative agents of tick-borne infections.

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ORIGINAL ARTICLE

PROGNOSTIC SIGNIFICANCE OF BLOOD MARKER OF HYPERTROPHY– CARDIOTROPHIN-1 WHEN CARRYING DIFFERENT VARIANTS OF ITS GENE IN MEN WITH ESSENTIAL HYPERTENSION

DOI:10.36740/WLek202102118

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ABSTRACT

The aim: To improve diagnosis of essential hypertension with left ventricular hypertrophy and chronic heart failure in men citizens of Podillya region in Ukraine by determining the plasma levels of cardiotrophin-1 in patients with different CT-1 gene variants.

Materials and methods: A total of 70 men with no signs of cardiovascular disease and 100 patients with essential hypertension were examined. Among those, 50 participants had hypertension and left ventricular hypertrophy. Other 50 patients had hypertension complicated by chronic heart failure.

Results: It was established that in patients with essential hypertension the frequency of the pool of genotypes GA + AA is higher than the genotype GG ($p < 0.05$). Plasma CT-1 levels ≥ 122,895 pg / ml can be used for early diagnosis left ventricular hypertrophy, and the cut-off level is ≥ 303.81 pg / ml (sensitivity 85.7%, specificity 92%) for screening diagnosis of essential hypertension complications in the form of chronic heart failure.

Conclusions: In patients with essential hypertension of varying severity, the GA+AA genotypes of CT-1 gene was found to be dominant. They had higher levels of plasma concentration CT-1. The threshold levels of CT-1 for screening diagnosis of essential hypertension with hypertrophy and chronic heart failure in males (who were residents of the Podillya region of Ukraine) were evaluated.

KEY WORDS: Cardiotrophin-1, essential hypertension, left ventricular hypertrophy, chronic heart failure, polymorphism of cardiotrophin-1 gene

Wiad Lek. 2021;74(2):273-277

INTRODUCTION

According to the Framingham study, the presence of left ventricular hypertrophy (LVH) doubles the incidence of cardiovascular events. An increase in LV wall thickness in patients with essential hypertension (EH) by 1 mm can be associated with an increased risk of dying by 7 times. The significance of myocardial hypertrophy as an independent predictor of cardiovascular complications in EH when assessing myocardial viability has been proved. [1,2].

At a certain stage of LVH development myocardial fibrosis and a number of other pathomorphological ones begin to form shifts, which ultimately lead to myocardial dysfunction and heart failure (CHF), which results in frequent hospitalizations and increased mortality of people with EH. This encourages the search for biomarkers for accurate diagnosis of LVH and prediction of CHF, especially in the early stages of the disease. Finding a solution to this problem requires an assessment of both the state of myocytes and myocardial connective tissue. One of the biomarkers that can help answer the above questions is cardiotrophin-1 (CT-1).

CT-1 is a member of the superfamily of cytokines interleukin IL-6 and is considered as one of the key regulators of hypertrophy and cardiomyocyte hyperplasia. CT-1 also affects the intensity of apoptosis and myocardial sensitivity to ischemia and collagen proliferation and secretion [3,4].

CT-1 releases its biological effects by interacting with the heterodimeric receptor gp130 and the receptor of leukemia inhibitory factor (LIFR), which causes intracellular activation of Janus kinase (Jaks) type I and II, as well as tyrosine kinase. It is shown that the expression of CT-1 is increased in response to stretching of the heart chambers and increased myocardial stiffness even before the increase in natriuretic peptides [5].

An important role in properties and the expression of CT-1 may belong to the polymorphism of the genes that encode it in particular at position rs8046707 G / A, and according to its biomarker value. Of particular interest is this aspect when using CT-1 not only as a biomarker of CHF, but also to clarify the state of the myocardium in such genetically dependent pathology as EH.

THE AIM

To improve diagnosis of essential hypertension with LVH and CHF in men citizens of Podillya region in Ukraine by determining the plasma levels of CT-1 in patients with different CT-1 gene variants.

MATERIALS AND METHODS

During the study we examined 170 middle-aged male residents of Podillya region. 100 men of the main group

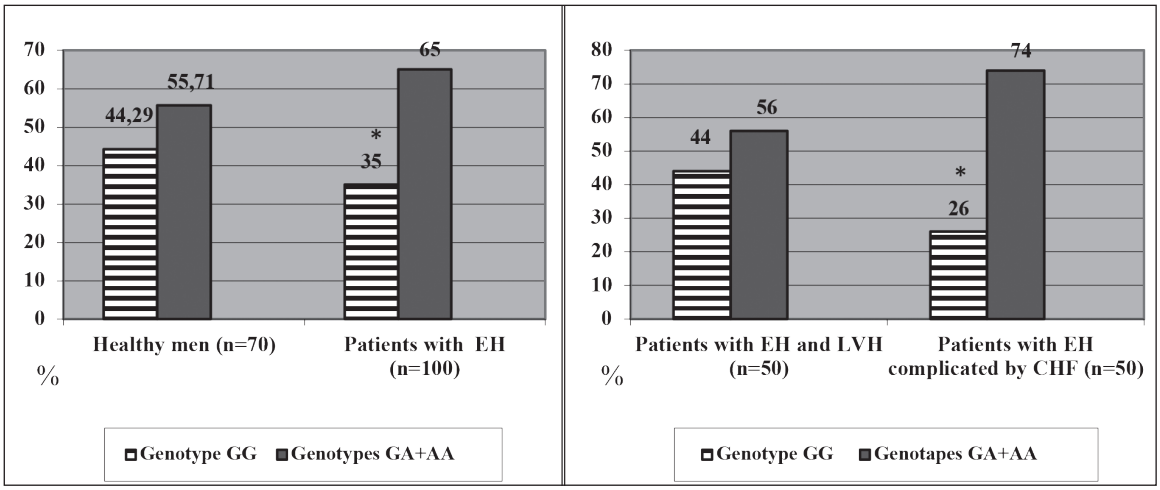

Fig. 1. The distribution of the CT-1 gene genotypes frequencies in men citizens of Podillya region in the healthy patients and the patients with EH and LVH and EH complicated by CHF (%).
Note: The difference is significant (p≤0,05) when compared to: * – GG genotype within each group.

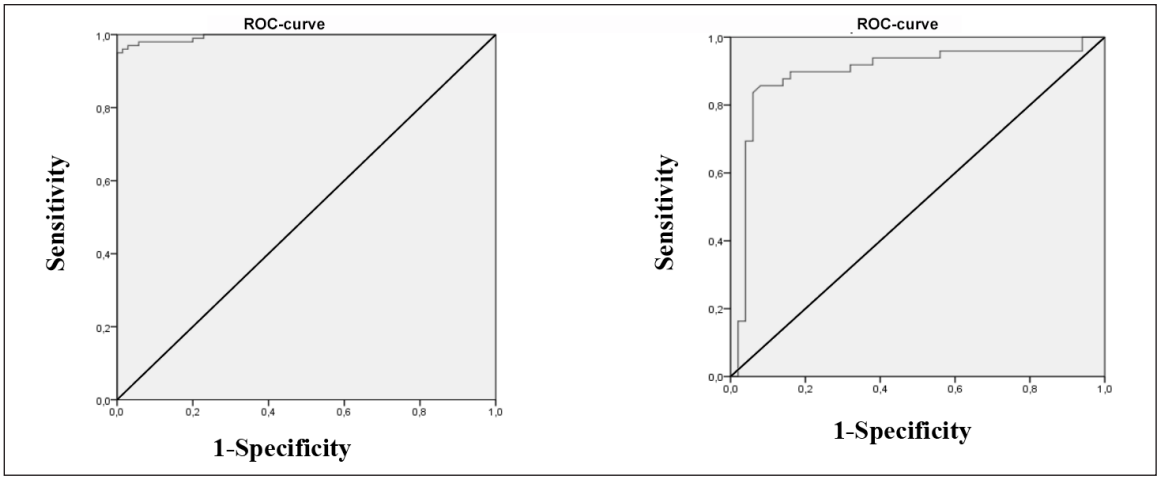

Fig. 2. ROC-curve to determine the cut-off level of CT-1 in blood plasma during the development of LVH in EH and EH complicated by CHF.

with EH with LVH, whose average age was 50,65±0,46 years. Among them, 50 men with EH with LVH (stages 1 and 2), with saved systolic function and CHF I-II classes according to NYHA Classification, whose average age was 50,62±0,73 and 50 men with EH complicated by CHF stage IIA, II-III classes according to NYHA Classification, whose average age was 51,86±0,81. 70 healthy men whose age was (48,81±0,78) did not differ from patients with EH and constituted the control group (p>0.05).

Exclusion criteria of the study were: secondary hypertension, renal and liver dysfunction, coronary heart disease the onset of which was before EH, endocrine, hematological, neoplastic and autoimmune disorders, patients with EH complications: myocardial infarction, acute cerebrovascular accident. These diseases were excluded

by collecting complaints, the results of an objective and general clinical examination (including, if necessary, pre-diagnosis of coronary heart disease), as well as a detailed analysis of outpatients' cards.

Genotyping of the CT-1 gene (rs8046707) was conducted using polymerase chain reaction (PCR) after isolation of genomic DNA from white blood cells of venous blood. This study was carried out jointly with the Research Institute of the genetic and immunological bases of pathology and pharmacokinetics "Ukrainian Medical Stomatological Academy" (Poltava, the head is prof. I.P. Kaidashev). The CT-1 concentration in plasma was determined by using ELISA method on enzymelinked immunosorbent analyzer "Humareader single» (Germany).

The mathematical processing was performed on a personal computer using a standard statistical package STATISTICA 10. Structural and functional parameters of the myocardium were evaluated using ultrasound of the heart.

RESULTS

The frequency distribution of the CT-1 gene genotypes in the men included in the study, residents of the Podillya region of Ukraine, corresponded to the Hardy-Weinberg equilibrium. Because of the relatively small number of patients with the AA genotype, we combined the patients with GA and AA genotypes in the carriers of the genotypes GA +AA.

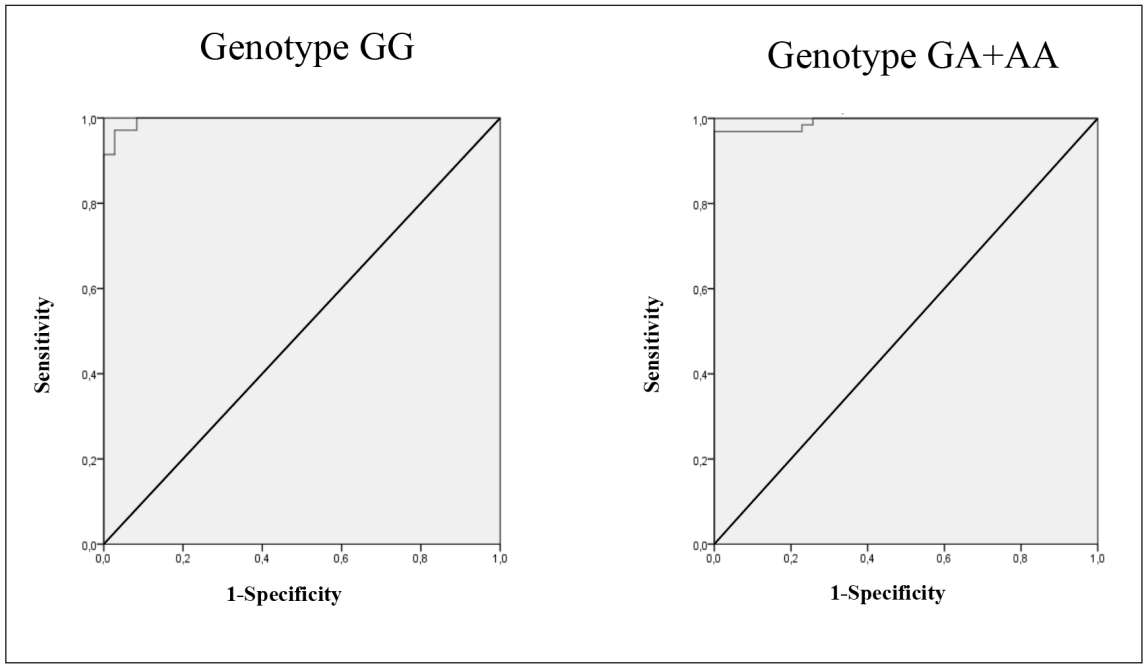

Fig. 3. ROC-curve to determine the cut-off level of CT-1 in blood plasma during the development of LVH in carriers of polymorphic variants of the CT-1 gene.

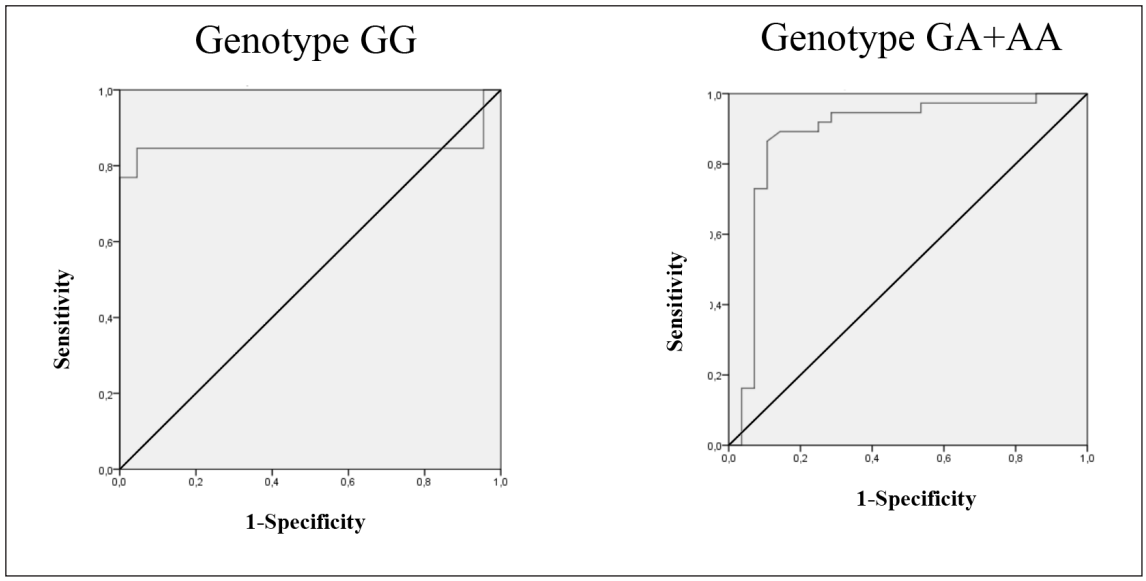

Fig. 4. ROC-curve to determine the cut-off level of CT-1 in blood plasma relative to the development of complicated EH, in carriers of polymorphic variants of the CT-1 gene.

In the control group, there were no significant differences in frequency carriage of the genotype variants of the CT-1 gene (p> 0.05).

In individuals with EH in general, the frequency of the GG genotype of the CT-1 gene was less than -35.00% (n = 65), which is below the pool of genotypes GA + AA -65.00% (n = 35) (p <0.05). However, in men with EH and LVH, the frequency of carriers of the GG genotype is 44.00% (n = 22), and the pool of genotypes GA + AA is 56.00% (n = 28) (p> 0.05). Among patients with EH complicated by CHF stage IIA, the frequency of GG genotype was 26.00% (n = 13), the pool of GA + AA genotypes was 74.00% (n = 37) (p <0.05). That is, among the studied contingent with EH reviews the pool of genotypes GA + AA of the CT-1 gene, at the expense of people with CHF. (Fig.1).

In the control groups of GG genotype owners, the main level of CT-1 in blood plasma was lower – 55.77 ± 2.53 pg/ml than the carriers of the pool of genotypes GA + AA – 92.46 ± 1.54 pg / ml and, accordingly, below in men with EH carriers of different genotypes of the CT-1 gene.

In men with asymptomatic EH and EH complicated by CHF, the plasma concentration of CT-1 is higher in carriers of the pool of genotypes GA + AA, respectively, 272.71 ± 12.57 pg/ml and 359.05 ± 5.79 pg/ml (p <0, 05) than in carriers of the GG genotype of the CT-1 gene (respectively 189.50 ± 9.51 pg/ml and 322.81 ± 27.01 pg/ml

Table 1. Indicators of ROC-analysis for the diagnosis of LVH and CHF in carriers of polymorphic variants of the CT-1 gene.

Group	«Cut off value»	AUC	p	Sensitivity, %	Specificity, %
LVH	122,89 pg / ml	0,995±0,003	p<0,05	95%	100%
LVH in carriers of the GG genotype	113,25 pg / ml	0,99±0,004	p<0,05	97,1%	98%
LVH in carriers of the GA+AA genotype	161,5 pg / ml	0,993±0,006	p<0,05	96,9%	100%
CHF	303,81 pg / ml	0,895±0,038	p<0,05	87,5%	92%
CHF in carriers of the GG genotype	266,955 pg / ml	0,850±0,096	p<0,05	84,6%	95%
CHF in carriers of the GA+AA genotype	323,32 pg / ml	0,884±0,050	p<0,05	86,5%	89,2%

(p <0.05)). Therefore, in men with the GG genotype and the GA + AA genotype with EH complicated by CHF, the level of the peptide is significantly higher than in persons without signs of cardiovascular pathology and in persons with asymptomatic EH (p <0.05).

The obtained data allowed us to calculate the boundary levels using ROC-analysis CT-1 in blood plasma for early diagnosis of LVH and CHF in men with EH, carriers of different variants of the genotype CT-1. This data can be used in the examination of large contingents of the population to identify persons who then need to conduct a full, including ultrasound examination of the heart and have appropriate treatment prescribed in cases of family examination (suspected hereditary pathology) and in expert cases, and in case of impossibility of instrumental examination because of various anatomical defects of the chest.

According to the Swets classification [6], the area under the ROC curve from 0.5 to 0.7 indicates low model accuracy, the model with the area under the ROC curve from 0.7 to 0.9 can be used in practice and the area under the ROC-curve above 0.9 characterizes highly accurate model. Based on the ROC-curve, which is presented in Fig. 2, sensitivity and specificity for different boundary points were calculated.

The area under the AUC curve according to ROC-analysis for the determination of CT-1 in blood plasma in the presence of EH and LVH of varying severity is 0.995 ± 0.003 [95% CI from 0.988 to 1.00;] which indicates the excellent quality of the obtained models. The cut-off point equal to ≥ 122,89 pg/ml 89% sensitivity and 92% specificity with an AUC equal to 93% This calculation allows to establish the cut-off level of CT-1 in blood plasma (sensitivity-95%, specificity-100%) in such a structural state of the myocardium as LVH. The area under the AUC curve according to ROC analysis for CT-1 is 0.895 ± 0.038 [95% CI from 0.822 to 0.969;], which indicates a very good quality of the obtained model. The obtained data indicate that the level of CT-1 in blood plasma ≥ 303.81 pg / ml (sensitivity – 85.7%, specificity-92%) can be considered as a boundary for the diagnosis of EH complicated by CHF.

However, it is also necessary to take into account possible deviations from the presented parameters in carriers of different variants of the genotype of the CT-1 gene, because the plasma concentrations of this biomarker in the respective subgroups differ.

The area under the AUC curve according to ROC analysis to determine the level of CT-1 in blood plasma in GG homozygotes is 0.99 ± 0.004 [95% CI from 0.988 to 1,000;], in carriers of the pool of genotypes GA + AA (Fig. 3) – 0.993 ± 0.006 [95% CI from 0.981 to 1,000;] which indicates the excellent quality of the obtained models. The cut- off CT-1 in blood plasma for the diagnosis of LVH in carriers of the GG genotype is ≥ 113,255 pg / ml (sensitivity – 97.1%, specificity-98%) and in carriers of GA+AA genotype ≥ 161.5 pg / ml (sensitivity – 96 , 9%, specificity – 100%).

The area under the AUC curve according to ROC analysis for the determination of CT-1 in blood plasma in carriers of the GG genotype is 0.850 ± 0.096 [95% CI from 0.662 to 1,000;], in carriers of the GA + AA genotype pool – 0.884 ± 0.050 [95% CI from 0.785 to 0.982;] (Fig. 4) which indicates a very good quality of the obtained model. These data indicate that in male homozygotes GG the level of CT-1 in blood plasma ≥ 266,955 pg / ml (sensitivity – 84.6%, specificity – 95%), and in carriers of the genotype GA + AA ≥ 323.32 pg / ml (sensitivity- 86.5%, specificity – 89.2%) allows to diagnose CHF.

The data presented in table 1 shows that the obtained results have a very good quality of the model, sensitivity and specificity for the diagnosis of LVH on the background of EH and CHF in men.

DISCUSSION

Thus, among 40 to 60 years old men, residents of the Podillia region in Ukraine who are patients with EH the frequency of registration of the pool of genotypes GA + AA was higher than in the group of patients with EH heterozygotes GG.

In a study done by Lutz S Z, Franck O et all. in the German population, it was found that the GA genotype is most common among Germans [7]. In both German and Ukrainian populations, the AA genotype is rarely identified.

In the study of plasma concentrations of CT-1 it was found that men with EH and LVH have significantly higher level those in the control group and in turn levels than persons with EH, complicated by CHF (p <0, 05). In men with EH of varying severity, the plasma concentration of CT-1 is significantly higher in carriers of the GA + AA genotype of the CT-1 gene. (p <0.05). In a meta-analysis conducted by K. Song, the plasma concentration of CT-1 not only increases with EH, but also has a prognostic value for the development of CHF [8]. In a study conducted by Kolesnik M.Yu. among the residents of Ukraine there was an increase in the concentration of the marker depending on the severity of impaired glucose metabolism. The results were such that in men with EH without impaired glucose metabolism, the concentration of the marker was 176.1 (106.5-436.2) pg / ml, in the presence of insulin resistance in combination with EH, the level of CT-1 in blood plasma 282.2 (119.5-650.2) pg / ml [9]. The study clarified the diagnostic limits for the confirmation of LVH in EH and CHF.

CONCLUSIONS

In 40-60 years old male residents of Podillya who were patients with EH the frequency of the pool of genotypes GA + AA is higher than the genotype GG (p <0.05) due to patients with CHF.

Plasma CT-1 levels ≥ 122,895 pg / ml can be used for early diagnosis of myocardial changes such as LVH, and the cut-off level is ≥ 303.81 pg / ml (sensitivity 85.7%, specificity 92%) for screening diagnosis of CHF.

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ORIGINAL ARTICLE

«OBESITY PARADOX» IN COMORBID STABLE ISCHEMIC HEART DISEASE AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS

DOI:10.36740/WLek202102119

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ABSTRACT

The aim: Was to find the possible relationship between spirometry tests, the BODE index (body mass index (BMI), airflow obstruction, dyspnea, and exercise tolerance) with bioimpedance parameters in overweight and class I obese patients.

Materials and methods: 47 patients with stable ischemic heart disease (IHD) (I-II functional class), chronic obstructive pulmonary disease (COPD) (GOLD II, III, IV; groups B, C, D) were divided into 3 groups: G1: 15 normal-weight patients, G2: 15 overweight subjects, and G3: 17 class I obese patients. Spirometry tests, bioimpedance parameters, 6MWT (6-minute walk test) were measured.

Results: FEV1 was significantly higher in overweight ($p = 0.033$) and class I obese ($p = 0.049$) subjects, the BODE index was lower in overweight ($p = 0.033$) and class I obese ($p = 0.037$) patients, compared with normal-weight subjects. The statistically significant positive relationship was between BMI and FEV1 and the negative correlation was between BMI and the BODE index in all groups of patients ($p < 0.05$).

Conclusions: In our study, we found better FEV1, 6MWT, the BODE index, the statistically significant association between FEV1 and the BODE index with BMI in overweight and class I obese patients. That's why we can suppose the presence of the "obesity paradox" in comorbid overweight or class I obese stable IHD, COPD patients.

KEY WORDS: COPD, obesity, spirometry

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INTRODUCTION

Nowadays, the impact of obesity on spirometry tests and quality of life in comorbid patients with stable IHD and COPD is very actuality [1,2,3]. Numerous studies have shown that low body BMI is an independent predictor of mortality in COPD patients [4]. Overweight or class I obese COPD subjects had a better prognosis and fewer number of exacerbations [1,2,3]. The results of large meta-analyses had shown the lower overall and cardiovascular mortality in overweight or obese stable IHD patients [5,6]. Therefore, the protective role of class I obesity on the spirometry tests and quality of life remains interesting, especially in comorbid stable IHD, COPD patients.

THE AIM

The aim of our study was to find the possible relationship between spirometry tests, the BODE index with bioimpedance parameters in overweight and class I obese patients.

MATERIALS AND METHODS

47 patients with stable IHD (I-II functional class), COPD GOLD II, III, IV; group B, C, D; were included in the study. The mean age was 62.22 ± 11.96. Among all patients, 55.32% were male and 44.68% – female. The diagnosis and stage of COPD were established according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations [7]. The diagnosis of stable IHD was performed in accordance with the European Society of Cardiology (ESC) guidelines [8]. Main inclusion criteria were: aged from 45 to 70, signed informed volunteer study consent, diagnosis of COPD with post-bronchodilator $FEV_1/FVC < 0.70$, grade II, III, IV of bronchial obstruction (according to GOLD 2010) and groups B, C, D (as to GOLD 2011) and the presence of stable IHD.

We divided all patients into 3 groups: G1: 15 patients with stable IHD, COPD, and $18.5 < BMI < 24.9$, G2: 15 overweight subjects ($25 < BMI < 29.9$), and G3: 17 class I obese patients ($30.0 < BMI < 34.9$). We used BTL – Spiro Pro computer spirograph (UK) to assess spirometry tests after a short-acting bronchodilator test (400 mg of salbutamol) in all groups of patients. We used a portable apparatus (TANITA BC-601, Japan) to determine all bioimpedance parameters. Also, all patients completed the CAT test and SGRQ to assess their overall health and quality of life. Dyspnea severity was performed using a Modified Medical Research Council Council (mMRC) scale. We used 6MWT to assess the submaximal level of exercise capacity. BMI, FEV_1, mMRC, and 6MWT were used to estimate the BODE

Table I. Baseline characteristics of comorbid stable IHD and COPD patients with different BMI categories

Group	Stable IHD, COPD, and 18.5 <BMI <24.9 (N = 15)	Stable IHD, COPD, and 25 <BMI <29.9 (N = 15)	Stable IHD, COPD, and 30.0 <BMI <34.9 (N = 17)	P value
male female	11 (87.5%) 4 (12.5%)	6 (40%) 9 (60%)	9 (52.94%) 8 (47.06%)	
Age (years)	67.53 ± 11.3	64.67 ± 10.75	55.94 ± 9.27	$p_3 = 0.007$
Pack-years, M ± SD	19.3 ± 15.53	6.67 ± 9.0	13,294 ± 16,26	$p_1 = 0.015$
GOLD II GOLD III GOLD IV group B group C group D	5 (33.3%) 6 (40%) 4 (26.7%) 5 (33.3%) 4 (26.7%) 6 (40%)	7 (46.66%) 4 (26.67%) 4 (26.67%) 7 (46.66%) 4 (26.67%) 4 (26.67%)	5 (29.41%) 10 (58.82%) 2 (11.77%) 5 (29.41%) 8 (41.18%) 5 (29.41%)	
BMI (kg/m^2)	22.6 ± 1.39	27.8 ± 1.35	33.66 ± 2.5	$p_1 < 0.0001$ $p_2 < 0.0001$ $p_3 < 0.0001$
body fat percentage	23.11 ± 5.7	32.83 ± 6.07	36.2 ± 9.69	$p_1 < 0.0001$ $p_3 < 0.0001$
muscle mass, kg	51.87 ± 7.88	49.15 ± 7.13	52.8 ± 8.59	NS
visceral fat level	9.13 ± 3.04	11.13 ± 4.3	17.65 ± 4.26	$p_2 < 0.0001$ $p_3 < 0.0001$
CAT score	23.2 ± 2.73	21.78 ± 1.64	22.12 ± 2.4	NS
SGRQ symptoms score	60.88 ± 9.58	57.24 ± 8.7	56.64 ± 7.55	NS
SGRQ activity score	50.48 ± 11.16	53.53 ± 13.31	47.31 ± 7.18	$p_2 = 0.033$
SGRQ impact score	42.87 ± 6.2	44.3 ± 4.56	43.21 ± 6.02	NS
SGRQ total score	55.1 ± 8.16	51.74 ± 6.2	52.83 ± 5.48	NS
post-bronchodilator, FEV_1	44.04 ± 11.56	52.78 ± 13.98	52.94 ± 12.45	$p_1 = 0.033$ $p_3 = 0.049$
mMRC score	2.27 ± 0.7	1.67 ± 1.05	2.0 ± 0.87	$p_1 = 0.033$
6MWT (meters)	230.33 ± 63.6	284.33 ± 71.83	287.35 ± 74.84	$p_1 = 0.023$ $p_3 = 0.018$
BODE index	5.00 ± 2.14	3.13 ± 2.6	3.47 ± 2.32	$p_1 = 0.033$ $p_3 = 0.037$
Modified BODE index	5.27 ± 2.66	3.73 ± 2.9	5.0 ± 2.24	$p_2 = 0.027$

Values are M ± SD or %; NS: not significant.
Note 1. p1 – the difference between stable IHD, COPD patients with 18.5 <BMI <24.9, and stable IHD, COPD patients with 25 < BMI <29.9.
Note 2. p2 – the difference between stable IHD, COPD patients with 25 <BMI <29.9, and stable IHD, COPD patients with 30.0 < BMI <34.9.
Note 3. p3 – the difference between stable IHD, COPD patients with 18.5 <BMI <24.9, and stable IHD, COPD patients with 30.0 < BMI <34.9.

index. The study meets the requirements of the Helsinki Declaration of the World Medical Association «Ethical principles for medical research involving human subjects as the object of study» opinion of the Committee on bioethics SHEI «Bukovinian State Medical University» of MPH of Ukraine №5/2020.

Statistical analyses were performed on a personal computer using SPSS Statistica 23. Quantitative data are presented as M ± SD, where M is the mean, SD – standard deviation. Normality was verified using the Shapiro-Wilk test. Non-parametric the Mann-Whitney U test was used to compare differences between each of two independent groups.

RESULTS

Clinical characteristics all groups of patients are presented in Table I.

We analyzed the results of bioimpedance analysis in all groups of patients. Body fat percentage was significantly

higher ($p < 0.0001$) in overweight or class I obese stable IHD, COPD patients compared with normal-weight subjects. There were no statistically significant differences between compared groups in muscle mass. The results of subjective assessment quality of life (CAT test, SGRQ) between all groups of patients were not statistically significant. The value of post-bronchodilator FEV_1 was significantly higher in overweight stable IHD, COPD patients ($p = 0.033$) and class I obese subjects ($p = 0.049$) compared with normal-weight patients. Also, overweight subjects had significantly lower mMRC score ($p = 0.033$) compared with the first group. There was no significant difference in the mMRC score between class I obese patients and normal-weight subjects ($p > 0.05$). The total distance walked in meters (6MWT) was significantly higher in the second ($p = 0.023$) and third ($p = 0.018$) groups of patients compared with the normal-weight subjects. Patients with normal BMI had significantly higher the BODE index compared with overweight ($p = 0.033$) and class I obese subjects ($p = 0.037$).

Also we found a statistically significant positive correlation between BMI and FEV_1 in the first ($r = 0.546$; $p = 0.035$), second ($r = 0.594$; $p = 0.02$) and third ($r = 0.738$; $p = 0.001$) groups of patients. The negative correlation was between BMI and the BODE index in the first ($r = -0.565$; $p = 0.028$), second ($r = -0.682$; $p = 0.005$) and third ($r = -0.568$; $p = 0.017$) groups. However, we didn't find a statistically significant correlation between the muscle mass and FEV_1 and the BODE index ($p > 0.05$).

DISCUSSION

Therefore, in our study, we have obtained results close to the data of numerous scientific publications about the role of overweight or class I obesity in stable IHD, COPD patients [9,10,11]. The inverse correlation was found between BMI and mortality in the meta-analysis of 22 studies involving 21150 COPD patients. Also, in this study overweight or obese (BMI ≥30) patients had a lower mortality rate compared to normal-weight subjects [9]. The results of another meta-analysis showed the impact of bodyweight deficiency and obesity on survival in COPD patients. Mortality rates were highest in bodyweight deficiency (BMI <21.75 kg/m2) subjects. Survival rates were the best in patients with BMI = 30 kg/m^2 [10]. Also, the results of 6MWT showed that, when obese COPD patients increased the distance on 1 meter, they had a better survival rate during the 26 months of the experiment [12]. The role of the BODE index is also very significant in assessing COPD patients. As to another study, the BODE index in class I obese patients was significantly lower compared with normal-weight subjects [13]. In our study, the BODE index was the highest in normal-weight patients compared to the second and third groups of patients. There was also a negative correlation between BMI and the BODE index. According to these studies, we can assume a better prognostic survival in overweight or class I obese subjects.

Also, in an 11-year study, involving 40,000 confirmed angiographic IHD subjects, the lowest mortality rate was in patients with 27.5 < BMI <30 kg/m2, but the presence of class III obesity increased mortality in twice [14]. In another study with 4,400 IHD participants was found, that obese patients (BMI >30), despite a large number of risk factors, had better 7-year survival rates compared to the normal-weight subjects [15].

As a summary, we concluded that the results of our study were similar to the data of previous scientific publications regarding the protective value of overweight or class I obesity in comorbid stable IHD, COPD patients. The value of post-bronchodilator FEV_1, 6MWT were significantly higher in the second and third groups compared to the first. The mMRC dyspnea severity and the BODE index were significantly lower in overweight or class I obese patients. Also, we noted the existence of a statistically significant positive relationship between BMI and FEV_1 and a negative correlation between BMI and the BODE index in all groups of patients. Previous researches had described the protective role of class I obesity or overweight separately in COPD patients and IHD subjects. But in our study the participants were comorbid. That's why maybe we can interpret the results of our study as the summary effect of the "obesity paradox".

CONCLUSIONS

Therefore, analyzing the literature and the results of our study, we can assume the "obesity paradox" in stable IHD, COPD patients. The value of post-bronchodilator FEV_1 and the prognostic survival score (the BODE index) in overweight or class I obese patients were specifically related to BMI. In our study, we did not find significantly higher muscle mass values in overweight or class I obese patients. But we noted a tendency to increase this parameter in class I obese patients. Maybe the results of the study will be another if the patients will perform muscle-strengthening exercises during some period with further assessment of spirometry tests and all bioimpedance parameters.

Nowadays, the main cause of the "obesity paradox" remains relevant and discussable in patients with chronic diseases.

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ORIGINAL ARTICLE

PRACTICAL MEANS OF PREOPERATIVE DIAGNOSTICS OF PRIMARY FALLOPIAN TUBE CANCER

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ABSTRACT

The aim: To analyze contemporary practical means to improve diagnostics of primary fallopian tube cancer.

Materials and methods: Authors analyzed specifics of clinical signs and anamnesis in 152 PFTC patients. Diagnostic capacity of cytological analysis of pathologic vaginal discharge, X-ray contrast methods of examination, sonography, tumor markers, and computed tomography was studied. Own results of PFTC diagnostics using different methods and world practice using MRI, PET-CT and laparoscopy were discussed.

Results: Using own observations authors conclude that clinical analysis and complex use of the listed methods allows to mainly determine high risk group patients and set correct preoperative diagnosis in 35% and preliminary diagnosis in 20% of PFTC patients.

Conclusions: Complex examination allows to recognize primary fallopian tube cancer on preoperative stage and to avoid inadequate surgical interventions in majority of PFTC patients.

KEY WORDS: primary fallopian tube cancer, complex examination, preoperative diagnostics

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INTRODUCTION

According to literature data and own observations, primary fallopian tube cancer (PFTC) amounts to no less than 1.5-1.9% of female genital cancers and 4-6% of cancers of uterine appendages. However, the true frequency of fallopian tube cancer is likely significantly higher, given that up until now, if neighboring organs are involved, this pathology is often considered being ovarian cancer [1 – 4]. Besides, epidemiological studies show increase in PFTC morbidity. Thus, from 2010 to 2014 morbidity in the USA increased 4-fold [5, 6]. Despite the improvement of diagnostic capacity of the contemporary methods of examination, accuracy of preoperative diagnostics of PFTC even in specific oncology centers still varies between 0% and 12-15% [2,4,7,8]. Diagnostic mistakes delay detection and complicate treatment of these patients. Thus, finding means of improvement of preoperative diagnostics of PFTC is a pressing medical issue.

THE AIM

Aim of the study was to conduct retrospective analysis of own long-term clinical observation and results of additional examination methods for further improvement in detection and effectiveness of preoperative diagnostics of PFTC, to review literature data and make practical conclusions.

MATERIALS AND METHODS

For the analysis we chose 152 PFTC patients with conclusive case histories. Age of the patients was between 34 to 78 years (mean age – 54.5±4.9 years). Age group from 46 to 65 years old contained 65% of PFTC cases. Diagnostic capacity of cytological analysis of pathologic vaginal discharge, X-ray contrast methods of examination, sonography, tumor markers, and computed tomography was studied. Own results of PFTC diagnostics using different methods and world practice using MRI, PET-CT and laparoscopy were discussed.

RESULTS

According to anamnesis and surgical revision data, around 70% of PFTC patients had pelvic inflammatory disease (PID) and 49% had infertility. All patients underwent surgical treatment and had diagnoses confirmed by histopathology. The majority of cases were serous carcinomas of different structure and differentiation (116 (76.3%)), second most common tumors were endometrioid (19 (12.5%)) and undifferentiated carcinomas (12 (7.9%)). Singular findings of clear cell, mucinous, and squamous carcinomas amounted to only 3.3% of cases.

In the study were analyzed specifics of clinical signs in primary fallopian tube cancer patients, and data of additional methods of examination in these patients on preoperative stage.

Previously performed analysis established that the reasons for delayed diagnostics of PFTC are mainly ignorance of clinical specifics of the tumor and insufficient cancer alert, and less – delayed seeking of medical care, hidden or atypical disease course [4].

The main clinical symptoms in most patients 114 (75 ± 3.5%) in the analysis, were the presence of secretions similar to lymphorrhea, bloody discharge in pre- and postmenopause, as well as the most typical sign of PFTC – hydrops tubae profluens 20 (13.2 ± 2.7%).

After seeking medical help during the bimanual examination, atypical changes of the appendages were palpated, which were confirmed during sonography, but could be interpreted as pathology of the uterus. Several women had a clinical picture of acute abdomen, which required emergency surgery in non-oncology departments. Diagnosis of PFTK was established only after histological examination.

Examination of women with adnexal masses, with or without suspicion of PFTC, on preoperative stage includes different additional investigational methods. One of the most available additional methods is cytological examination of pathological uterine discharge.

To make a preoperative diagnosis, we make extensive use of X-ray contrast techniques (hysterosalphingography (HSG) and bicontrast HSG), which often allow PFTC to be diagnosed at an early stage. According to the analysis, the correct diagnosis was in 62 (82 ± 4%; CI: 71-90) patients, in contrast to the use of ultrasound scanning. Only in 2 (13.3%) cases USS showed a typical picture of PFTK.

An additional method in the complex diagnosis of PFTK are tumor markers, namely CA-125. Although its diagnostic accuracy is very controversial [2,3,8]. In our analysis, we concluded that CA-125 is not accurate enough in the early stages, but is a significant early marker of cancer recovery. Diagnostic laparoscopy should be considered as an intraoperative diagnosis. However, its advantages, features and disadvantages require further in-depth study.

DISCUSSION

Many authors studying primary fallopian tube cancer report difficulties of its preoperative diagnostics: some of them blame oligosymptomatic nature of the disease, others blame low specificity of the symptoms [2,3,4,7]. Diagnostics of the disease is challenging even during laparotomy, which is proven by reports of 30-50% of incorrect diagnoses during surgery [4,9,10]. Most often it happens in patients in the early stages of the disease who undergo surgery in non-oncological profile hospitals without suspicion of malignization, which leads to nonradical surgery and delay, or even sometimes absence, of adequate treatment. Up-to-date some authors consider diagnosis of PFTC as an intraoperative finding, others – as pathoanatomical rather than clinical diagnosis [2,11,12]. Even in a specialized oncological institution correct diagnoses of PFTC during surgery were made only in 47.5% cases [7].

114 (75±3,5%) patients with PFTC complained of different kind of pathological discharge from sexual pathways. In most cases discharge were liquid and opalesque, of yellowish or amber colour, mainly lymphorrhea-like, with constant or periodic blood admixture noticed in up to half of those patients. The second most common discharge type were bloody spotting in postmenopause, beginning bloody spotting in premenopause was commonly considered being an acyclic uterine bleeding. 20 (13,2±2,7%) patients had the most typical sign of PFTC – hydrops tubae profluens, a condition when profuse watery vaginal discharge happens during colicky pain episode which leads to pain relief and decrease in size or even disappearance of a previously palpated tubo-ovarian mass. More rarely patients (5.2%) experienced vaginal discharge of variable color or yellowish discharge with purulent admixture. Typical for all types of vaginal discharge in PFTC is their common connection with pain syndrome, persistent increase and ineffectiveness of different treatment measures including uterine diagnostics curettage. Rarely discharge are mild and can be diagnosed only on colposcopy or during sonography, when fluid in uterine cavity is found. 107 (70,4±3,7%) patients with PFTC complained of pain. Around a third of them had dull sometimes colicky pain in the lower abdomen on an affected side, rarer like renal or tubal colic. In cases of aggravation of concomitant chronic inflammatory process, torsion of affected tube or its perforation some patients (6 (3,9%)) developed "acute" abdomen. In cases of disseminated tumors involving neighboring organs patients complained of different types of pain and other signs, including weakness, fatigue, fever, discomfort and bloating in abdomen, dysuria, etc.

According to patients' histories and intraoperation revision data, around 70% of patients with PFTC had pelvic inflammatory disease and 49% complained of infertility.

Upon seeking medical care, 116 (76,3±3,4%) PFTC patients already had anatomical changes of internal genitals, which were revealed on bimanual examination. In more than half of the patients enlargement of appendages were palpated as ovoid, sausage-like or irregular formation of uneven, more often rubber elastic consistency, sometimes mobile and almost painless. Almost every fifth patient had unclear changes in appendages, which were interpreted as pathology of uterus. As opposed to ovarian cancer, implantation metastases and tumor infiltrates in rectouterine pouch are not common in PFTC. All this signs and clinical findings, while grouped into symptom complexes, created variety of clinical courses of PFTC, often masked as other, no less dangerous diseases.

It is worth to mention that 10 (6,5±1,9%) PFTC patients prior to diagnosis considered themselves healthy and had no complaints. In them the disease was found on routine examination, including 4 cases found on routine sonography or while diagnosing another pathology.

However, according to our observations, 25 (16,4±3,0%) patients had typical for PFTC classic clinical triad decribed by Latzko in 1916 [4,12]. It includes waterish, lymph like, discharge related to pain syndrome, often hydrops tubae profluens, and palpated (or found on ultrasonography) characteristic masses in the areas of adnexa.

Quite typical clinical picture was present in 23 (15,1±3,3%) patients. They had one or two typical symptoms and other less significant signs (anamnesis, age, treatment ineffectiveness), which allowed to suspect PFTC and use more informative additional diagnostic methods.

PFTC, clinically proceeding similar to endometrial cancer, was found in 23 (15,1±2,9%) patients. Majority of those patients underwent, sometimes even repeatedly, dilation and curettage, however, groups of tumor cells were found in 2 cases and typical endometrial cancer – in 1 case. The latter was endometrial cancer synchronous to fallopian tube cancer (histopathological and immunohistochemical analyses revealed serous papillary adenocarcinoma in uterine tube and highly differentiated endometrioid adenocarcinoma in uterus). 28 (18,4±3,1%) patients were diagnosed with different adnexal masses on ultrasonography, mostly thought to be ovarian cysts or hydrosalphinx, suspicion of malignancy was stated if papillary outgrowth was present. 12 (7,9±2,1%) PFTC patients were hospitalized with initial diagnosis of leiomyoma of uterus, while a part of them really had it, and some were observed and treated previously. There were cases, where typical for PFTC sonographic picture with normal ovaries was considered to be a subserosus pedunculated fibroid with signs of necrosis.

Clinical picture of "acute" abdomen was present in 6 (3,9±1,5%) patients, all of them had emergency surgery in non-oncology departments. Half of those patients had incomplete radical operations and diagnosis of PFTC was established only after histopathological study.

14 (9,2±2,3%) PFTC patients had prolonged treatment for pelvic inflammatory disease, often with temporary subjective improvement. In them PFTC imitated PID, or PID was a concomitant process. Diagnostically challenging situation was present in 9 (5,9±1,9%) young women, in whom PFTC masked as abnormal uterine bleedings, and in 2 (1.3±0,9%) PFTC patients an extragenital tumor was suspected prior to laparotomy.

Examination of women with adnexal masses, with or without suspicion of PFTC, on preoperative stage includes different additional investigational methods. One of the most available additional methods is cytological examination of pathological uterine discharge. Probability of receiving positive result in patients with discharge is 75%. Although according to literature data, accuracy of cytological results varies between 0% and 40%. It is explained by hardship of obtaining cytological sample of sufficient quality and quantity [13-16]. We specifically examined 95 PFTC patients using different methods of sampling (vaginal and cervical smears; uterine aspirate; discharge on cervical cap; discharge sampled on hysterosaplingography). Different methods provided accurate preoperative results in 11%-65% of cases. The least informative were routine vaginal and cervical smears. That is why we recommend to use a combination of sampling methods [17].

Starting from the 70s and 80s we have widely used X-ray contrast methods (hysterosalphingography (HSG) and bicontrast HSG). Among 76 PFTC patients examined by cytology and X-rays, 44 (58±6%; CI: 46-69) patients received accurate preoperative diagnosis, together with suspicious for PFTC cases, the correct diagnosis was present in 62 (82±4%; CI: 71-90) patients. HSG should still be included into diagnostic spectrum. For instance, US authors consider that this method allows correction between MRI and sonography. In 2005 visual atlas of HSG was published [4,18]. We still periodically use HSG, and this method, used together with other methods, often allows for early stage PFTC diagnostics [17].

Ultrasound scanning (USS) is often used in gynecology for diagnostics of different pathology and sometimes as screening due to patient's wish. In 2006 we have originally analyzed USS results on preoperative stage in 15 PFTC patients. Only in 2 (13,3%) cases USS showed typical picture of PFTC, and experienced clinician could suspect and state correct diagnosis. Many authors report the similar [18,20]. But technical progress leads to improvement in diagnostics, especially after introduction of transvaginal and doppler energetic scanning. During the last decade we performed USS on preoperative stage in 43 PFTC patients. Out of them 8 (19±6%; CI: 8-33) patients received accurate or preliminary diagnosis of PFTC, and 9 (21±6%; CI: 11-37) patients had quite typical clinical picture, suspicious for PFTC. Therefore, only sonography already allowed for accurate clinical diagnosis in 17 (40±8%; CI: 25-56) patients. 8 (18,6±6%) patients from the same group received ultrasonic diagnosis of ovarian cancer, 7 (16,3±5,6%) patients received diagnosis of cyst or papillary cyst of ovaries, 8 (18,6±6%) patients – of sactosalphinx or other adnexal masses, and only in 3 patients no pathology was found. Alas, ultrasonic signs of PFTC are not always specific and can imitate tubal pregnancy, tuboovarian abscesses, tuberculosis of adnexa or another pathology [7,19,21].

Literature data suggest that magnetic resonance imaging (MRI) and positron emission tomography (PET) are often used for PFTC diagnostics [1,22,23]. According to some authors these methods not only visualize a tumor and its structure, but unlike USS, allow to recognize fine details of contrast, of hydrophilic and chemical specifics of tissues, to identify tumor infiltrates, their borders and metastases in regional lymph nodes [23-25]. There are even suggestions to include MRI into FIGO guidelines as a compulsory diagnostic procedure for PFTC [24].

We were unable to find current literature data on preoperative diagnostics of PFTC using computed tomography (CT). Out of our 12 PFTC patients who underwent CT, and 6 patients who underwent MRI, only 1 patient received clear description (on spiral CT) of a "109x47x54 mm mass in the projection of a left fallopian tube", which together with clinical and USS data allowed to state accurate diagnosis on a preoperative stage. Examination results in other patients were not sufficiently informative.

Tumor markers are also used in complex diagnostics of PFTC. Literature data mainly suggests CA-125, but reports of its diagnostic accuracy are very contradictory [2,3,8]. According to many separate observations and our data, levels of CA-125 are usually normal or dubious in the early stages of PFTC, especially if concomitant chronic diseases are present [4,11,23]. After radical surgery or chemotherapy,

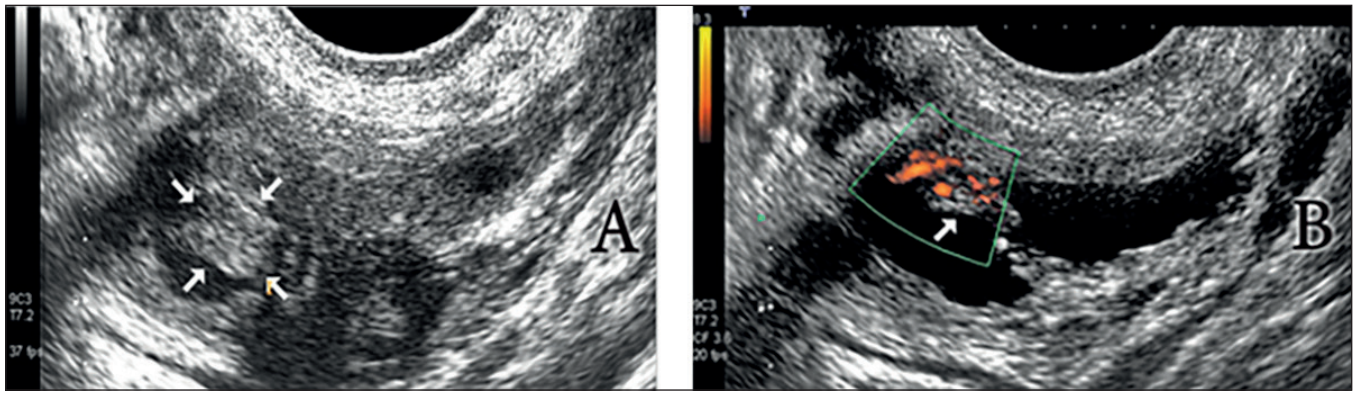

Fig. 1. Sonography scans of the patient.
A – multiple papillary outgrowth on the inner wall of fallopian tube (pointed by arrows).
B – on Doppler energetic (colorful) scanning presence of moderate intensity hypervascularisation with increased speed and decreased resistance in the same area of fallopian tube (pointed by arrow).

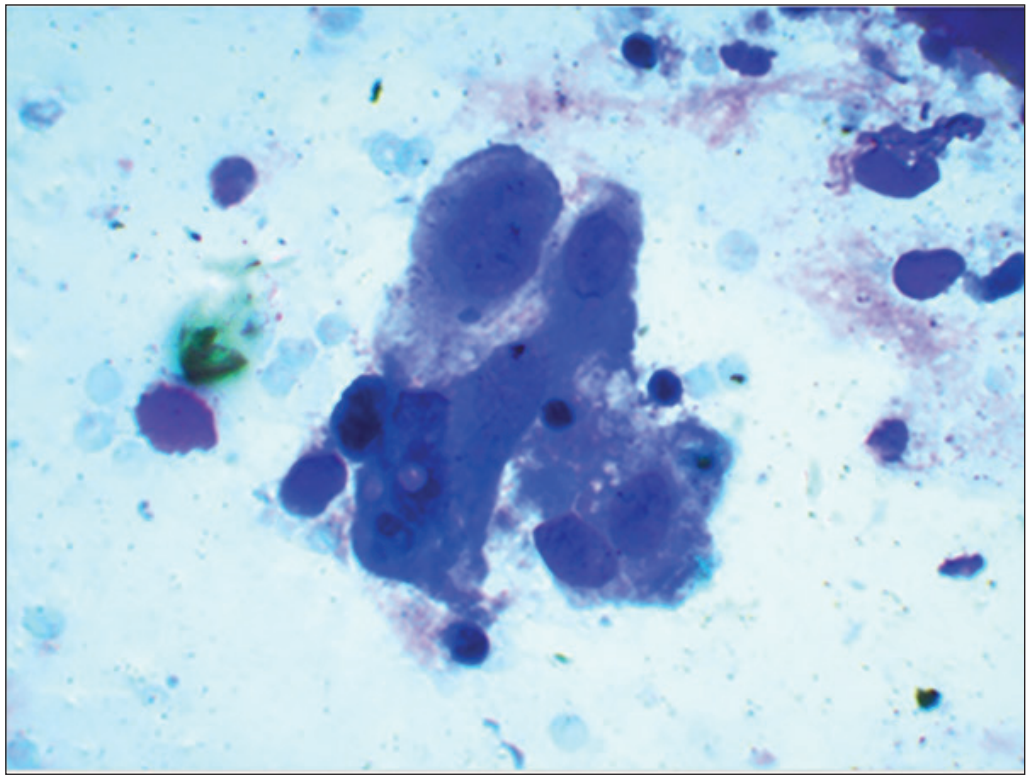

Fig. 2. The patient's uterine cavity aspirate. Photo of micropreparation. Romanowsky–Giemsa staining. x400 enlargement.

level of CA-125 normalizes, and increases again in cancer reactivation [8,11]. This allows for 3 months earlier diagnostics of disease recurrence compared to clinical-laboratory or X-ray methods [3,8,11]. We analyzed results of CA-125 testing on different stages of diagnostics and treatment in 30 PFTC patients, our conclusion is that CA-125 is not sufficiently accurate on the early stages, but is a significant early marker of a cancerous process reactivation [4].

CASE REPORTS

In order to demonstrate practical means of PFTC diagnostics, we would like to provide one of our latest case reports.

Patient B, 49 years old, addressed a gynecologist with complaints of lower abdominal pain. On bimanual examination – slight pain on shifting of uterus; on colposcopy – a drop of amber liquid in cervical canal, lymphorrhea was suspected. Immediately after doctor performed uterine aspiration for cytological test, patient was referred to USS. Sonography revealed bilateral hydrosalpinx, on the left with papillary structures 6.2x2.9 mm with strong blood flow of increased speed and low resist; impression of probably malignant neoplasm in distal part of left fallopian tube (Fig.1).

Cytological analysis of uterine aspirate revealed elements of glandular cancer (Fig.2). Other examinations results: CT – small fibroids in uterus, ovaries are normal, left side hydrosalpinx; CA-125 – 14,69 U/ml (normal ≤ 35,0 U/ml); HE-4 – 104 pmol/l (normal ≤ 74,3 pmol/l). Preoperative diagnosis: left sided PFTC.

Patient underwent radical hysterectomy with adnexa, pelvic and lower lumbar lymph nodes, and greater omentum resec-

tion. Histopathological analysis: serous adenocarcinoma of left fallopian tube without serous layer invasion, metastases in right fallopian tube and right upper iliac lymph nodes. Final diagnosis: Primary fallopian tube cancer T2A N1 Mo.

A group of cells of mucous epithelium with considerable polymorphism of nuclei. Nuclei contain 1-2 nucleoli, fine chromatin.

Diagnostic laparoscopy should be regarded as intraoperative diagnostics. According to R.Wenzl at al. the odds of finding PFTC in general laparoscopic practice is 1 per 3687 laparoscopies or 0.028% [4]. Literature search showed only case reports on diagnostic laparoscopy in PFTC, without detailed elaboration of diagnostic specifics [23,27,28]. Considering prolonged 'closed nature' of PFTC and masked progression as hydro- or hematosalpinx, there are very low chances of recognizing on laparoscopy without histopathological analysis a primary fallopian tube cancer without peritoneal spread. Spread of cancer cells from punctured or reopened fallopian tube to pelvic cavity considerably worsens patient's prognosis. Patrick F. Timmins et al. report decrease of 10-years survival from 58% to 7% if cancer cells are found in abdominal cavity [29]. Indications, advantages and specifics of laparoscopic diagnostics of PFTC should be studied further.

CONCLUSIONS

The current analysis of anamnestic and clinical specifics in PFTC patients proves that proper oncologic alertness and complex focused use of the methods, mentioned in the article, allows for accurate suspicion and correct preoperative diagnostics in PFTC patients, leading to referral to specialized medical centers. During years 2015-2019 there were 23 cases of PFTC in our region. Out of them 3 patients were operated in non-specialized hospitals, including 1 patient operated in another region. In 2 of them oncologic process wasn't recognized on operation. The remaining 20 out of total 23 PFTC patients had complex examination and surgery in regional clinical oncologic dispensary. Accurate preoperative diagnosis was set in 7 (35±11%) patients, preliminary diagnosis – in 4 (20±9%) patients. Three (15±8%) patients has preoperative diagnosis of endometrial cancer, because elements of the tumor were found on diagnostic dilation and curettage. One of them had simultaneously endometrial cancer and PFTC. In the other 6 (30±10%) patients adnexal masses were recognized as ovarian cancer or papillary cyst suspicious for malignancy. Mainly, PFTC was diagnosed on preoperative stage in the major half of patients. Though, we must stress that correct diagnoses were set mainly basing on the clinical signs of the disease.

We consider the following important:

– if pathological discharge are present, especially in women of high risk groups, several methods of cytological sampling should be used at once;
– if adnexal mass is visualized on sonography, further examination should be carried out using transvaginal scanning in energetic Doppler mode with special attention to the character of contents, capsule, structure of the mass, and especially blood flow;
– if necessary, use MRI, PET-CT and X-rays contrast methods of diagnostics;
– puncture of is adnexal masses is unacceptable, including during laparoscopy;
– if PFTC is found on operation and there is no possibility to invite oncogynecologist, the patient should be transferred to oncogynecology department directly after surgery. Detailed description of revision of abdominal and retroperitoneal structures should be provided for adequate staging and determination of necessity of radical surgery or other methods of treatme

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ORIGINAL ARTICLE

THE ROLE OF THE KIDNEYS IN THE REGULATION OF THE ACID – BASE BALANCE OF THE BLOOD IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

The aim: To study the regulation of acid-base balance and blood acid – renal excretory function in patients with COPD.

Materials and methods: We examined 82 people, suggests that even during the most severe stages of COPD. Group 1 included 56 patients with COPD, group C. The average age was 60.54 + 2.04 years old, including 24 men and 32 women. The second group included 16 patients with COPD, group B, whose average age was 55.37 + 3.21 years old, including 7 men and 9 women. The third group included 10 healthy individuals, with an average age of 34.30 + 2.21 years, including 6 men and 4 women. Respiration function was evaluated on the basis of the forced expiratory curve recorded on a Spirolab II MIR S / N computer spirograph. The following indicators were evaluated: forced vital capacity (FVC), forced expiratory volume (FEV1) and FEV1 / FVC ratio.

Results: For all patients with COPD there is a characteristic presence of acidosis (pH in patients with COPD group B – 7,34 ± 0,01, in patients with COPD group C – 7,31 ± 0,07). For patients with COPD group C there are pronounced respiratory disorders (pCO_2 – 48,25 + 1,14 mm Hg, $p0_2$ – 28.07 +1.37 mm Hg). For patients with COPD group B characteristic metabolic disorders (BE--3,71 + 0,57), which increase as the disease progresses. For patients with COPD group C this figure is equal to 7.62 + 0.49. Thus, the analysis of indicators indicates the presence for all patients of mixed (respiratory and metabolic) acidosis, which increases as the chronic obstructive pulmonary disease progresses.

Conclusions: There is activation of acid – renal excretory function and the inclusion of renal mechanisms in the regulation of acid-base balance.

KEY WORDS: chronic obstructive pulmonary disease, chronic kidney disease, the acid-secreting function of the kidneys, acid based balance

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INTRODUCTION

According to statistics, the prevalence of COPD among adults is about 4-6% [1]. If COPD ranked 6th among the causes of death in 1990, then next year they are projected to occupy the 3rd place. In the age group over 45, COPD is now ranked 4th among the causes of death. Within 3 years after hospitalization of patients with COPD for exacerbations, the overall mortality rate is 49%. In this regard, timely diagnosis and effective treatment of COPD has become an increasingly urgent problem in modern pulmonology [2, 3]. Chronic obstructive pulmonary disease (COPD) and chronic kidney disease (CKD) affect a large number of patients. The World Health Organization estimates COPD to become the 3rd leading cause of mortality worldwide in 2030 [1]. CKD, defined by abnormalities of kidney structure or function for more than 3 months [2], affected 14.8% of the U.S. adult general population in 2011–2014 [3]. Cigarette smoking and increasing age are risk factors for the development of both COPD and CKD [4–6], with systemic inflammation as an extrapulmonary manifestation of COPD potentially increasing the risk of comorbid CKD [7]. This combination of COPD and CKD is independently associated with a higher prevalence of other comorbidities (especially cardiovascular) and increased mortality [8, 9].

Cardiovascular dysfunction is a well-known predictor of a limited functional capacity and health status [10]. Whether CKD and kidney function have a role for functional limitations independent of established cardiovascular disease is currently unknown.

THE AIM

To study the regulation of the acid-base balance of the blood and the acid-secreting functions of the kidneys in patients with COPD group B and C.

MATERIALS AND METHODS

In the Pulmonology Department of Kharkiv Regional Hospital, we examined 82 people, which are divided into 3 groups. Group 1 included 56 patients with COPD, group C. The average age was 60.54 + 2.04 years old, including 24 men and 32 women. The second group included 16 patients with COPD, group B, whose average age was 55.37 + 3.21 years old, including 7 men and 9 women. The third group included 10 healthy individuals, with an average age of 34.30 + 2.21 years, including 6 men and 4 women.

Respiration function was evaluated on the basis of the forced expiratory curve recorded on a Spirolab II MIR S / N

Table I. Clinical characteristics of patients.

Diagnosis / indicators	COPD gr. B	COPD gr. C
Dyspnea	100,00±0,28	100,00±3,01
Cough	100,0010,28	100,00+3,01
With sputum	54,5+15,02	50,00± 15,07
Fever	18,2± 13,64	0
Asthma attack	87,5±11,69	100,00±3,01
Dry rails	100,00+0,28	100,00±3,01
Bandbox sound due to percussion of the lung	62,5+17,11	54,50±5,01
X ray of respiratory organs: deformation a pulmonary drawing, increasing of transparency of lungs	50,00±17,67	36,40±4,50

Table II. Indicators of respiratory function in patients with COPD.

Diagnosis / indicators	Control group	COPD gr. B	COPD gr. C
	n= 10	n = 16	n = 56
FVC (%)	94,0013,30	80,2314,05*	57,53+2,42 **
FEV_1 (%)	102,2014,50	74,1515,14*	44,42+3,24 **
FEV_1 / VC (%)	105,0010,06	73,32+5,67*	69,4713,39 **

* – p <0.05 compared with the control group

** – p <0,05 when comparing the indicators with the control group and COPD

computer spirograph. The following indicators were evaluated: forced vital capacity (FVC), forced expiratory volume (FEV_1) and FEV_1 / FVC ratio.

The following indicators of gas and acid-general composition of venous blood were studied using Bayer 348 gas analyzer: pH, partial pressure of carbon dioxide (pCO_2) and oxygen ($p0_2$), plasma bicarbonate ($HC0_3$), deficiency or excess of buffer bases (BE).

The acid-secreting function of the kidneys was investigated by measuring the excretion of titratable acids (E t.a.), the excretion of ammonium (E_{MH4}) and the determination of excretion of hydrogen ions (E n +).

RESULTS AND DISCUSSION

As can be seen from table 1, all patients with COPD had shortness of breathing and cough. Half of the patients had cough accompanied by sputum. An increase in body temperature was observed in 18.20% of patients with COPD, group B. In the group of patients with COPD, group C body temperature was within normal range. In 100% of patients with COPD, group C, and in 87.5% of patients with COPD, group B asthma attacks were noted.

In 62.5% of patients with COPD, group B and in 54.5% of patients with COPD, group C in the percussion of the lungs a bandbox sound was noted. During auscultation of the lungs, the presence of rigid breathing and crackling wheezing was noted in all examined patients, both with COPD, group B and C. Due to X- ray examination, deformation of the pulmonary pattern were noted in 75.00% of patients with COPD, group B and 81.80% in patients with COPD, group C. Increased lung transparency was observed in 50.00% of COPD, group B and 36.40% of COPD, group C in accordance.

The results of the study of the function of external respiration are presented in table 2. As can be seen from table 2, for patients with COPD, group B compared to the control group, there is a marked decrease in forced lung capacity (FVC), forced expiratory volume (FEV_1), and FEV_1 / VC.

In the group of patients with COPD group C recorded 44.42 + 3.24%. Thus, function indicators further reduce these indicators. Thus, FVC external respiration in all patients indicate a significant reduction to 57.53 + 2.42%, FEV_1 ventilatory disorders, which often leads to an increase in the disproportionate ratio of ventilation / overload and gas exchange disorders.

Indicators of acid-base status of blood in these patients are presented in table 3. For all patients with COPD there is a characteristic presence of acidosis (pH in patients with COPD group B – 7,34 ± 0,01, in patients with COPD group C – 7,31 ± 0,07). For patients with COPD group C there are pronounced respiratory disorders (pCO2 – 48,25 + 1,14 mm Hg, p02 – 28.07 +1.37 mm Hg). For patients with COPD group B characteristic metabolic disorders (BE-- 3,71 + 0,57), which increase as the disease progresses. For patients with COPD group C this figure is equal to 7.62 + 0.49. Thus, the analysis of indicators indicates the presence for all patients of mixed (respiratory and metabolic) acidosis, which increases as the chronic obstructive pulmonary disease progresses.

At these stages, COPD shows active involvement of the kidneys in the compensatory processes, as evidenced by the indicators in Table. 3. Participation of kidneys in compensation of acidosis in patients with COPD is carried out at the expense of statistically significant increase in excretion of hydrogen ions (in patients with COPD group B -109.95 + 10.12 mmol / dl). The increase in the excretion

Table III. Acid-alkaline state of blood and acid-secreting functions of kidneys in patients with COPD

Diagnosis / indicators	Control group n = 10	COPD gr. B n = 16	COPD gr. C n = 56
	7,40±0,01	7,34+0,01*	7,31+0,07
BE mk/l	0,36+0,01	-3,71+0,57 *	-7,62+0,49**
pO_2, mm Hg	39,30+4,30	41,11+4,37 *	28,07+1,37**
pCO_2, mm Hg	39,20±0,15	4.1,70+2,72 *	48,25+1,14**
Et.a. mmol/dl	27,52±0,33	33,62+2,62	36,15+2,60 **
E_{NH4}, mmol/dl	47,33+0,057	61,23+3,97*	73,81+7,52
E н+, mmol/dl	74,85+6,59	94,85+0,42*	109,95+10,12*

* - p <0.05 compared with the control group
** - p <0,05 when comparing the indicators with the control group and COPD

of hydrogen ions in both groups of patients is due to the increased excretion of titratable acids and ammonium. It should be noted that the highest excretion of titratable acids (36,15 + 02,60 mmol / d) and ammonium (73,81 + 7,52 mmol / dl) takes place in the COPD group C. The analysis of these indicators shows that even at the most difficult stages of COPD, there is an activation of the acid-secreting function of the kidneys and the involvement of the renal mechanisms in the regulation of acid balance.

CONCLUSIONS

1. For patients with COPD characterized by severe ventilatory disorders, which are a decrease in the rate of forced lung capacity, forced expiratory volume and FEV_1/VC.
2. Indicators of *acid-base balance* reflect the degree of progression of COPD and are characterized by signs of respiratory, metabolic and mixed acidosis.
3. The kidneys are actively involved in the regulation of CLS in patients with COPD group B and COPD group C by increasing the excretion of titratable acids and ammoniogenesis.

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ORIGINAL ARTICLE

PECULIARITIES OF THE MORPHOMETRIC PARAMETERS OF SUPRAHYOID REGION OF THE HUMAN PREFETUSES

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ABSTRACT

The aim: To determine the peculiarities of the morphometric parameters of suprahyoid region of the human prefetuses.

Materials and methods: Thirty specimens of human prefetuses of 14.0-80.0 mm parietococcygeal length (PCL) (7-12 weeks of IUD) were studied using a complex of modern methods of morphological research.

Results: On the basis of obtained digital indicators of the main morphometric parameters of human SHR in the dynamics of the prenatal period of IUD the critical periods of development of the region were clarified and mathematical functions that describe the normal course of organogenesis of SHR were created, which can be useful for creating diagnostic algorithms for the norm when carrying out prenatal diagnostics and monitoring the state of the fetus. It has been established that the 9-10th week of IUD is a critical period in the development of SHR, since during this time, intensive growth processes occur, which are manifested by a sharp change in the size of the organ, and this can lead to the appearance of variants of the structure and possible congenital defects of the SHR and the dental-maxillary apparatus in general.

Conclusions: 1.Age-depended dynamics of changes in the anterior angle of the SHR shows an almost linear decrease in the angle by the end of the 9th week of IUD almost to 76°, after which it increases to almost 90° by the end of the 10th week. From the 11th week of the IUD, the anterior angle decreases again to 77 °, but begins to increase at the 12th week and by the end of the prefeal period. 2.The lateral length of SHR increases almost uniformly until the 9th week of IUD, during which its growth rate slows down. Starting from the end of the 10th week of IUD, this morphometric parameter begins to grow rapidly until the end of the prenatal period of ontogenesis. The growth rate of the lateral length of the SHR is described by the function: $L\ lat = 1.1025 + 0.0015\ x + 0.001\ x^2$. 3.The width of the SHR from the 10th week of IUD begins to grow rapidly until the end of the prenatal period of development. The growth rate of the width of SHR is described by the function: $W = 1.1025 + 0.0015\ x + 0.001\ x^2$. 4.Analysis of the age dynamics of the area of SHR demonstrates the exponential dependence on the age of the prefetuses, which is described by a mathematical function: $A = 1{,}2452\exp(0{,}0424x)$. Meanwhile, there is a slight slowdown in its growth rate at the 10th week of IUD with subsequent recovery of growth by the end of the prenatal period of ontogenesis. 5.The 9-10th week of IUD is a critical period in the development of SHR, since during this time, intensive growth processes occur, which are manifested by a sharp change in the size of the mandible.

KEY WORDS: anterior cervical region, suprahyoid triangle of the neck, prefetus, prenatal ontogenesis, human

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INTRODUCTION

Finding out the features of morphogenesis and topographic changes of suprahyoid region (SHR) of the neck and its structures in the dynamics of human intrauterine development (IUD) remains a relevant area of morphological research [1-5]. Refined, comprehensive data on gender, age and constitutional features of the structure and topography of organs and structures of the SHR of the neck during the prenatal period of human ontogenesis will make it possible to develop new criteria for the interpretation of medical diagnostic imaging data, the degree of fetal viability, improve existing and develop new methods of surgical correction of congenital neck defects [6-9].

THE AIM

To determine the peculiarities of the morphometric parameters of suprahyoid region of the human prefetuses.

MATERIALS AND METHODS

Thirty specimens of human prefetuses of 14.0-80.0 mm parietococcygeal length (PCL) (7-12 weeks of IUD) were studied using a complex of modern methods of morphological research: anthropometry, morphometry, three-dimensional reconstruction and statistical analysis. The morphometric parameters of SHR were determined: width (distance between the inner surfaces of the angles of the mandible), anteroposterior length (distance from the anterior surface of the hyoid bone to the lower edge of the mental symphysis), lateral length (length of the lateral border of the SHR – distance from the inner surface of the angle of mandible to the lower edge of the mental symphysis), the anterior angle of the SHR (the angle between the lateral borders of the SHR). The area of the supralingual area was determined by the morphometric parameters of the sides and the width of the SHR.

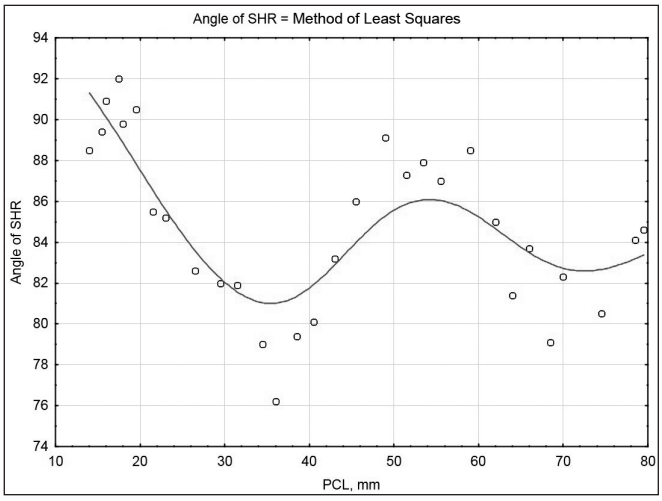

Fig. 1. The dynamics of changes of the anterior angle of the suprahyoid region in the prenatal period of human ontogenesis. Method of Least Squares.

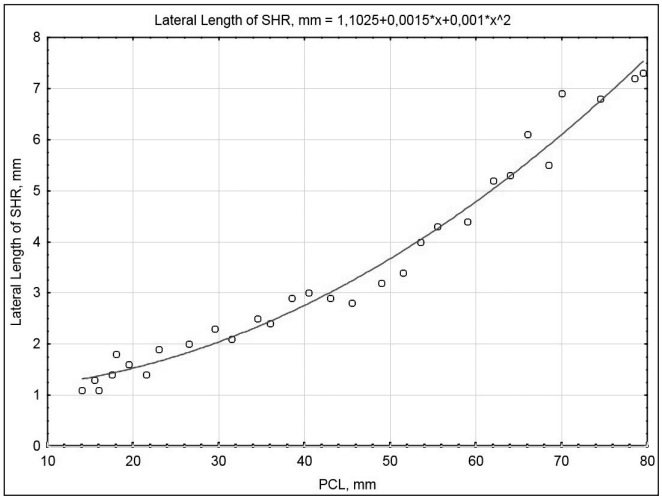

Fig. 2. Dynamics of changes in the lateral length of the suprahyoid region in the prenatal period of human ontogenesis. Polynomial dependence on age.

RESULTS

Analysis of the dynamics of morphometric parameters of the SHR of the neck allows to determine the features of their temporal changes, their possible unevenness during the prenatal period of human ontogenesis. Each of the parameters affects the area of the SHR, which is close to triangular in shape, and the size of the anterior angle and the width of the SHR are interrelated in a particular person – the greater the angle, the greater the width of the SHR, and vice versa. The clinical and diagnostic value of the area, which can be determined during ultrasound, is to predict malformations and variants of the structure of the mandible and anterior cervical region. Normative indicators of the size of the SHR and its area in the different terms of the prefetal period of IUD are morphological basis for the development of diagnostic algorithms for the interpretation of medical diagnostic imaging data. On the basis of obtained digital indicators of the main morphometric parameters of human SHR in the dynamics of the prenatal period of IUD the critical periods of development of the region were clarified and mathematical functions that describe the normal course of organogenesis of SHR were created, which can be useful for creating diagnostic algorithms for the norm when carrying out prenatal diagnostics and monitoring the state of the fetus.

Analysis of the age dynamics of changes in the anterior angle of the SHR by the method of Least Squares shows an almost linear decrease in the angle by the end of the 9th week of IUD almost to 76°, after which it increases to almost 90° by the end of the 10th week (Fig. 1). From the 11th week of the IUD, the anterior angle decreases again to 77 °, but begins to increase at the 12th week and by the end of the prefeal period. In our opinion, this feature of fluctuations in the magnitude of the anterior angle during the prenatal period of ontogenesis is explained by the processes of structural rearrangement of Meckel's cartilage during the development of bone tissue and the formation of the mandible.

The lateral length of SHR increases almost uniformly until the 9th week of IUD, during which its growth rate slows down (Fig. 2). Starting from the end of the 10th week of IUD, this morphometric parameter begins to grow rapidly until the end of the prenatal period of ontogenesis. We explain the deceleration in the growth of the lateral length of the SHR at the 9th week of IUD by the end of the main processes of mandibular formation (intensive ossification of the bony base of the organ on the cartilaginous model, which is reduced during this period).

The growth rate of the lateral length of the SHR is described by the function:

$L\ lat = 1.1025 + 0.0015\ x + 0.001\ x^2$,

Where L lat is the lateral length in mm,

x – PCL in mm.

The width of the SHR is the distance between the inner surfaces of the angles of the mandible, measured along an arcuate line that passes in front of the body of the hyoid bone. Analysis of the dynamics of changes in this parameter of the IHR shows a polynomial dependence on the age of the prefetuses (Fig. 3). At first there is a moderate growth rate of this parameter, from the 10th week of IUD the width of the SHR begins to grow rapidly until the end of the prenatal period of development. We believe that this feature of age morphometric dynamics is associated with structural changes in the mandible, which last until the 10th week, after which there are mainly processes of organ growth.

The growth rate of the width of SHR is described by the function:

$W = 1.1025 + 0.0015\ x + 0.001\ x^2$,

Where W is the width of SHR in mm.

x – PCL in mm.

Analysis of the age dynamics of the area of SHR demonstrates the exponential dependence on the age of the prefetuses, which is described by a mathematical function:

$A = 1{,}2452\exp(0{,}0424x)$,

Where A is the area of SHR in mm^2,

x – PCL in mm.

Meanwhile, there is a slight slowdown in its growth rate at the 10th week of IUD with subsequent recovery of growth by the end of the prenatal period of ontogenesis.

Thus, it can be argued that the 9-10^{th} week of IUD is a critical period in the development of SHR, since during this time, intensive growth processes occur, which are manifested by a

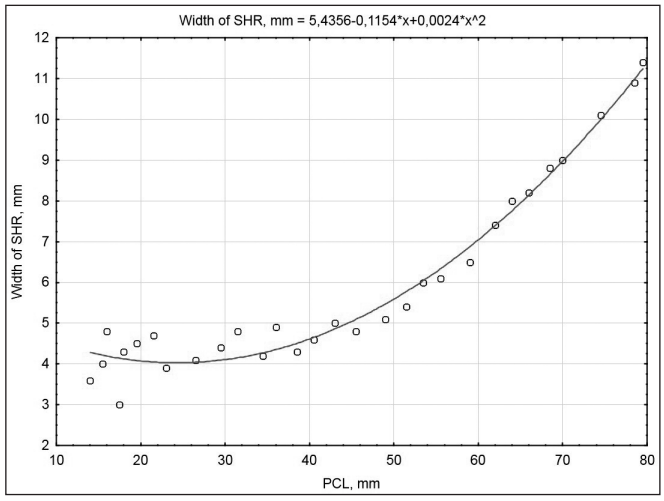

Fig. 3. Dynamics of changes in the width of the suprahyoid region in the prenatal period of human ontogenesis. Polynomial dependence on age.

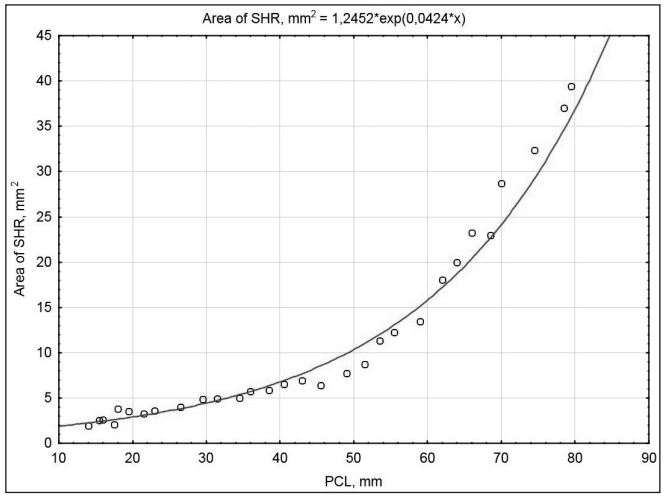

Fig. 4. Dynamics of changes of the area of the suprahyoid region in the prenatal period of human ontogenesis. Exponential dependence on age.

sharp change in the size of the mandible, and this can lead to the appearance of variants of the structure and possible congenital defects of the SHR and the superior neck region in general.

DISCUSSION

In our study the development of the structures of the suprahyoid region of the neck successively in the age-related dynamics of prenatal development has been studied by means of a complex of morphological methods. The method of 3D reconstruction of a series of consecutive histological sections of the head and neck, microscopic examination of our material and morphometry of suprahyoid triangles was widely used.

The majority of scientific studies of prenatal development of the suprahyoid triangles is limited to a certain age period. We have investigated the development of the SHR during a fairly long and important period of human development – from 7th to 12th weeks. It is during this period that complex processes of morphogenesis of the mandible and neck muscles, formation of definitive topographic-anatomical relationships take place, which is emphasized by other researchers in this area [1, 3, 10, 11]. A characteristic feature of the development of suprahyoid muscular triangles of the neck is the uneven growth of their morphometric parameters, due to the different growth rates of the structures that limit it. The morphometric parameters of the SHR were studied at different times by different methods of medical diagnostic imaging, as well as by the method of preparations and measuring the specimens [9, 11, 12, 13, 14]. Particular attention should be paid to the periods of slow or accelerated growth of morphometric parameters of the SHR found by many researchers. Scientists believe that it is during periods of uneven growth of structures that are in close syntopic connection, it is possible to develop variants of the structure of the SHR, as well as the occurrence of congenital malformations [14, 15]. Therefore, we paid attention to the peculiarities of the growth of such indicators as the dynamics of changes in the anterior angle, the length of the lateral border, the width and area of the SHR. Analysis of spatio-temporal changes in morphometric parameters allowed to derive mathematical functions that describe the normal course of morphogenesis in the prenatal period of IUD, and their comparison on a time scale allowed to determine possible morphological causes of certain morphometric features of morphogenesis.

In our opinion, the age-speciphic dynamics of changes in the morphometric parameters of the SHR in human prefetuses depends primarily on the peculiarities of the development of the mandible and suprahyoid muscles during this period, as indicated in other studies [16, 17].

Radlanski R.J. et al. [10] note that at the beginning of the prefetal period of IUD, and in fact, at the end of the embryonic period – in embryos of 11.0-12.0 mm PCL (end of the 6th week of IUD) – all muscles are clearly differentiated and enlarged by size. By the beginning of the 7th week of IUD, the Meckel's cartilage is already bent upwards and the places of attachment to their lower edge of the mylohyoid, geniohyoid та mylohyoid muscles [18]. At this age, the muscles of the suprahyoid group acquire definitive attachment sites due to the intensive development of the osseous model of mandible and the gradual involution of the Meckel cartilage [19]. In our opinion, it is the intensity of these morphologically opposite processes can significantly affect the shape of the mandible, and hence the morphometric parameters of the whole region.

We have identified critical periods of SHR development, which are predominantly 9-10 weeks of IUD. Radlanski R.J. et al. [10, 11], Levihn W.C. [13] also reported a significant increase in the size of the maxilla and mandible between the second and third trimesters of gestation, and therefore, in fact, the corresponding changes in the growth rates of morphometric parameters of the suprahyoid cervical triangles.

The mathematical functions obtained by us, which describe the normal course of organogenesis of the SHR, can be useful for the creation of diagnostic algorithms of the norm in the prenatal diagnosis of malformations of the neck and head, as well as for monitoring the condition of the fetus.

CONCLUSIONS

1. Age-depended dynamics of changes in the anterior angle of the SHR shows an almost linear decrease in the

angle by the end of the 9th week of IUD almost to 76°, after which it increases to almost 90° by the end of the 10th week. From the 11th week of the IUD, the anterior angle decreases again to 77°, but begins to increase at the 12th week and by the end of the prefeal period.
2. The lateral length of SHR increases almost uniformly until the 9th week of IUD, during which its growth rate slows down. Starting from the end of the 10th week of IUD, this morphometric parameter begins to grow rapidly until the end of the prenatal period of ontogenesis. The growth rate of the lateral length of the SHR is described by the function: $L\ lat = 1.1025 + 0.0015\ x + 0.001\ x^2$.
3. The width of the SHR from the 10th week of IUD begins to grow rapidly until the end of the prenatal period of development. The growth rate of the width of SHR is described by the function: $W = 1.1025 + 0.0015\ x + 0.001\ x^2$.
4. Analysis of the age dynamics of the area of SHR demonstrates the exponential dependence on the age of the prefetuses, which is described by a mathematical function: $A = 1{,}2452\exp(0{,}0424x)$. Meanwhile, there is a slight slowdown in its growth rate at the 10th week of IUD with subsequent recovery of growth by the end of the prenatal period of ontogenesis.
5. The 9-10th week of IUD is a critical period in the development of SHR, since during this time, intensive growth processes occur, which are manifested by a sharp change in the size of the mandible.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

HEALTH DYNAMICS OF THE MEDICAL UNIVERSITY STUDENTS DURING SPORTS ACTIVITIES

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ABSTRACT

The aim: Is to investigate the dynamics of the morphofunctional development and physical health of students who were engaged in strength sports while studying at university.

Materials and methods: The study involved 360 male students of different faculties between the ages of 17 and 20. Two groups of students were formed: experimental and control groups. The EG students (n=40) were engaged in strength sports (powerlifting, athletics, Crossfit); the CG students (n=320) were training according to the current program of physical education. The study of the morphofunctional development of students was carried out taking account of the indicators of body length, body weight, handgrip test, heart rate, blood pressure, and vital capacity. The level of students' health was examined according to the methodology of the assessment of the physical health level by G. L. Apanasenko.

Results: It was established that strength sports at university affect physical development, functional abilities of the major systems of an organism, and the health state of future doctors in a more efficient way than the current program of physical education. It was found that the influence on the indicators of handgrip test, vital capacity, and heart rate of the EG students was the most prominent positive effect of sports. The evaluation of the calculated indexes (power index, life index) and the level of physical health confirmed this trend.

Conclusions: The conducted research asserts the necessity of introducing the sports-oriented form of the physical training organization at the medical higher education institutions of Ukraine to strengthen the students' health and to maintain the efficiency of the future doctors' professional activity.

KEY WORDS: health, sport, physical education, students

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INTRODUCTION

The system of modern medical higher education is aimed at training a highly qualified specialist who is able to perform significant amounts of work without reducing the quality and intensity and must have a high level of psychomotor qualities that reflects the requirements for physical and mental health, physical fitness and working efficiency of students, future doctors [1, 2]. However, the analysis of the scientists' research results [3, 4, 5] showed a low level of physical fitness of most modern students and a constant increase in the number of people with impaired health both at the beginning and in the process of studying at Ukrainian higher education institutions (HEI).

It is generally accepted that human health is the exclusive competence of health authorities. However, it is a known fact that medical sciences study the diagnosis of diseases, the treatment process, and the means of disease prevention. Many other sciences, including physical education, solve the problems of health promotion, physical and mental development, study the problems aimed at expanding the functional capabilities of students [6, 7, 8].

The works of scientists [9, 10] note that the biological age of the students between the ages of 20 and 24 gets ahead of the stated age by 10-15 years. The studies of G. L. Apanasenko [11] determine that the share of the male population in the "safe zone" of health has decreased from 8 to 1 % over the last 20 years in Ukraine. The works of other scientists [13, 14] mention that the number of students with low and below the middle level of somatic health increased from 60 % in 2009 to 85 % in 2019. The scientists [15, 16] point out that some factors of life and socially hygienic living conditions, as well as studying at HEI do not provide optimal health for students. As a result of students testing, scientists [17] defined the initial level of physical fitness of the first-year students, among whom 18 % showed a low level; 49 % – lower than the middle (unsatisfactory grade); 20 % – the middle level (satisfactory grade); 13 % – above the middle (good grade). No students with a high level of physical fitness were found.

The research many scientists [17, 18] notes that a significant part of student does not reach even the middle level of physical fitness during the study that indicates a low effi-

ciency of the physical education system in Ukraine. Among the main reasons for the decrease in the physical fitness of students, the authors highlight the lack of health-promoting and training orientation of the forms and means of physical education, low motor activity of students, the lack of interests, motives, and needs for physical exercises, etc. [19, 20]. Some studies [21, 22] also mention that the decline in the health indicators of students goes back to the health of school students and university applicants. The basics of health should be laid at school age, however, according to scientists, the number of healthy school graduates accounts for 5-25 %, more than 50 % of them have unsatisfactory physical fitness, 90 % of school graduates have health problems.

The World Health Organization defined approximate ratios of various factors that ensure and form the health of modern people, namely: genetic factors (heredity) – 20 %, the state of the environment (climate, environmental circumstances) – 20 %, the level of health care (medical care) – 8 %, living conditions and lifestyle (rational work, physical activity, nutrition, personal hygiene, rejection of bad habits) – 52 % [23]. According to many scientists [14, 15, 24], the conditions and way of life, with various elements that relate to all aspects of health – physical, mental, social, and spiritual, are the key elements of improving the health and efficiency of students.

Physical exercises are of great importance for health promotion and disease prevention [18, 20, 25]. World experience shows that the physical activity of a person throughout life prevents diseases and improves health. However, according to scientists [3, 4, 10], the difficult economic situation, developed in Ukraine in the post-Soviet period, negatively affected the development of physical education and mass sports at HEI. Every year the number of students training in special medical groups is steadily growing, the number of healthy students is decreasing. Scientists note that under such conditions, the formation of a healthy lifestyle, and the involvement of young students in regular exercises and sports should become one of the important areas of the HEI's activities. Scientists [18, 20, 26] consider the introduction of a sports-oriented form of physical education of students taking into account the students' sports choice a promising direction for improving the system of physical education at HEI. At the same time, the popularity of the sport among students, the possibilities of the educational and sports base of HEI, and the specialists among the teaching staff of the Department of Physical Education should be taken into account.

THE AIM

The aim is to investigate the dynamics of the morphofunctional development and physical health of students who were involved in strength sports while studying at university.

MATERIALS AND METHODS

The study of the morphofunctional development and physical health of students was conducted at I. Horbachevsky Ternopil National Medical University (Ukraine) in 2018-2020. The study involved 360 male students of different faculties between the ages of 17 and 20. Two groups of students were formed: an EG (n=40) and a CG (n=320). The EG students belonged to the sports educational department and were engaged in such strength sports as powerlifting, athletics, Crossfit in specialized sports clubs at university. The CG students belonged to the main educational department and were training according to the current program of physical education. To investigate the morphofunctional development of university students, we analyzed the following indicators: body length, body weight, handgrip test, heart rate, systolic and diastolic blood pressure, and vital capacity. The level of students' health was examined according to the methodology of the assessment of the physical health level by Professor G. L. Apanasenko [11]. The health level was evaluated in points and it included the estimation of the body mass index (BMI – the ratio of body weight to body length), life index (LI – the ratio of vital capacity to body weight), Robinson's index (RI – a product of heart rate and systolic blood pressure), power index (PI – the ratio of the indicators of handgrip test to body weight) and heart rate recovery (HRR) after a standard exercise (20 squats in 30 sec) (Table I). The research was conducted in the 1st – 4th terms of study. Medical examinations were conducted by the doctors of medical center of the university.

The methods of investigation: analysis and generalization of the scientific and methodological literature, observation, pedagogical testing, medical and biological methods, and methods of mathematical statistics. The authenticity of the difference between the indicators of students of EG and CG was determined by Student's t-test and the dynamics of the indicators during studying were examined.

RESULTS

The analysis of the students' body length showed that a significant difference between the indicators of the EG and CG was not detected at all stages of the study ($p>0.05$). During the 1st and 2nd years of study, the body length of students in both groups increased, but no significant difference between the indicators of the 1st and 2nd years was found ($p>0.05$) (Table II). This indicates that both classes according to the current program of physical education at medical universities, and classes in the specialized sports clubs of strength sports do not affect the body length of the HEI students significantly.

The study of the body weight dynamics shows that in the 1st – 3rd terms of study, the indicators of the EG and CG students did not differ significantly ($p>0.05$). In the 4th term, the body weight of the EG students was significantly lower than that of the CG students by 2.9 kg ($p>0.05$). A comparative analysis of the students' body weight at the beginning and at the end of the study showed that it increased significantly by 3.7 kg in the CG ($p<0.001$) and did not change authentically in the EG ($p>0.05$) (Table II). It proves the positive impact of sports

Table I. The physical health level evaluation according to the methodology by G. L. Apanasenko (male, points)

The indicators investigated	The level of physical health				
	Low	Below the middle	Middle	Above the middle	High
BMI, kg/m^2	18.9 i <	19.0-20.0	20.1-25.0	25.1-28.0	28.1 i >
Numerical score	-2	-1	0		
LI, ml/kg	50 i <	51-55	56-60	61-65	66 i >
Numerical score	*-1*	*0*	*1*	*2*	*3*
PI, %	60 i <	61-65	66-70	71-80	81 i >
Numerical score	*-1*	*0*	*1*	*2*	*3*
RI, s.u.	111 i >	95-110	85-94	70-84	69 i <
Numerical score	*-2*	*-1*	*0*	*3*	*5*
HRR, sec	180 i >	120-180	90-120	60-90	59 i <
Numerical score	-2	1	3	5	7
The amount of points	3 i <	4-6	7-11	12-15	16-18

Table II . Comparative characteristics of the EG and CG students' physical development indicators while studying at a medical university (Mean±SD)

Terms of study	EG (n=40)	CG (n=320)	Significance value
Body length, cm			
1st	176.1±1.25	175.9±0.77	p>0.05
2nd	176.4±1.24	176.3±0.75	p>0.05
3rd	176.5±1.24	176.4±0.74	p>0.05
4th	176.9±1.23	176.5±0.72	p>0.05
Body weight, kg			
1st	72.7±1.36	72.5±0.59	p>0.05
2nd	73.1±1.41	73.8±0.45	p>0.05
3rd	73.2±1.28	75.0±0.52	p>0.05
4th	73.3±1.29	76.2±0.54	p<0.05
Handgrip test, kg			
1st	38.9±1.20	38.6±0.72	p>0.05
2nd	41.3±1.18	39.5±0.68	p>0.05
3rd	43.2±1.16	39.9±0.65	p<0.05
4th	46.5±1.15	40.4±0.66	p<0.001
Vital capacity, ml			
1st	3982.4±97.11	3960.7±52.13	p>0.05
2nd	4144.1±96.84	4038.3±48.20	p>0.05
3rd	4295.9±92.58	4097.5±47.74	p>0.05
4th	4427.3±89.30	4155.4±47.22	p<0.05

Legend: Mean – arithmetical average; SD – standard deviation; p – the significance of difference between the indicators of EG and CG due to the Student's t-test

on the physical development of medical students. The established trend of the CG students' weight gain shows that the current conditions of studying at HEI, which are characterized by large amounts of educational material, long-lasted staying in classrooms in a position, low motor activity, nervous and emotional stress, especially during exams sessions, lead to the body weight gain. At the same time, classes according to the current methodology for physical education do not effectively influence the stabilization of students' body weight. The body weight of the students who regularly attend specialized clubs in strength sports at university remains stable during the period of study.

The study of the handgrip test showed that the strength of the arm muscles of the students of both groups did not differ significantly in the 1st and 2nd terms of study (p>0.05) (Table II). The indicators of the EG students' handgrip test started to exceed significantly the indicators of the CG students from the 3rd term: by 3.3 kg in the 3rd term (p<0.05) and by 6.1 kg in the 4th (p<0.001). During

Table III. Comparative characteristics of the EG and CG students' functional state indicators while studying at a medical university (Mean±SD)

Terms of study	EG (n=40)	CG (n=320)	Significance value
Heart rate at rest, beats/min			
1st	71.0±1.15	70.9±0.52	p>0.05
2nd	69.6±1.13	70.9±0.51	p>0.05
3rd	69.3±1.12	71.2±0.53	p>0.05
4th	68.7±1.10	71.3±0.53	p<0.05
Systolic blood pressure, mmHg			
1st	120.2±1.09	120.1±0.42	p>0.05
2nd	120.1±1.06	120.0±0.42	p>0.05
3rd	119.9±1.06	120.2±0.41	p>0.05
4th	119.8±1.05	120.2±0.42	p>0.05
Diastolic blood pressure, mmHg			
1st	70.5±0.63	70.9±0.37	p>0.05
2nd	70.2±0.62	70.8±0.36	p>0.05
3rd	69.8±0.60	70.7±0.36	p>0.05
4th	69.6±0.60	70.7±0.35	p>0.05

Legend: Mean – arithmetical average; SD – standard deviation; p – the significance of difference between the indicators of EG and CG due to the Student's t-test

the research, the muscle strength of the students of both groups increased, but indicators improved significantly in the EG (p<0.001), and insignificantly in the CG (p>0.05). It indicates by far better effect of the classes in specialized clubs in strength sports on the physical development of students – future doctors.

The analysis of the vital lung capacity showed that a significant difference between the indicators of the EG and CG students was not detected in the 1st – 3rd terms of study (p>0.05). It was recorded only in the 4th term – 271.9 ml (p<0.05) (Table II). The investigation of the vital capacity dynamics indicates that the indicators of the respiratory system significantly improved during training in both groups, but the difference between the indicators of the 1st and 4th terms was 444.9 ml in the EG (p<0.001), and 194.7 ml in the (p<0.05) that proves the positive effect of sports on the functional abilities of the respiratory system of students.

The analysis of resting heart rate shows that a significant difference between the indicators of the EG and CG students was not detected in the 1st – 3rd terms of study (p>0.05). In the 4th term, the heart rate of the EG students was 2.6 beats/min better than that of the CG students authentically (p<0.05) (Table III). During the study, the heart rate of the students of both groups tended to improve. However, the indicators improved by 2.3 beats/min in the EG, and only by 0.4 beats/min in the CG (p>0.05).

The study of blood pressure points put that the indicators of both systolic and diastolic pressure were the same for the EG and CG students authentically in the 1st-4th terms of study (p>0.05) (Table III). During the research, the blood pressure of the EG students had a tendency to improve, but the indicators did not change authentically (p>0.05).

The analysis of body mass index stated no significant difference between the indicators of the EG and CG students during the whole study (p>0.05) (Table IV). The investigation of the dynamics of the body mass index shows that body mass index was deteriorating in the CG, the worst value was recorded in the 4th term (24.20 kg/m^2). The index was stable in the EG during all terms of study that confirmed the positive influence of sports, in contrast to classes according to the current program of physical education. The evaluation of the body mass index proved that in accordance with Table I, the index of the students of both groups was within normal limits. However, given the negative dynamics of the body mass index of the CG students, it can be argued that this index will continue to deteriorate in the senior years of studying.

The analysis of the life index showed that no significant difference between the indicators of the EG and CG students was found in the 1st and 2nd terms of study (p>0.05). The life index of the EG students was significantly better than that of the CG students by 4.94 ml/kg (p<0.05) in the 3rd term and by 6.88 ml/kg (p<0.001) in the 4th term (Table IV). During the study period, the life index of the EG students improved by 5.68 ml/kg authentically (p<0.05), and the index of the CG students worsened not authentically (p>0.05). According to Table I, the life index in the CG was assessed as below the middle at all stages of the study, and in the EG it was assessed as the middle in the 1st – 3rd terms, and above the middle in the 4th term. It indicates a positive impact of sports on the performance of students' respiratory systems.

The analysis of the power index showed that the power index in the EG and CG did not differ authentically in the 1st and 2nd terms of study (p>0.05). In the 3rd term, the power index of the EG students was significantly better in comparison with the CG students' by 5.78 % (p<0.05), in the 4th term – by 10.37 % (p<0.001) (Table IV). During

Table IV. Comparative characteristics of the EG and CG students' physical health while studying at a medical university (Mean±SD)

Terms of study	EG (n=40)	CG (n=320)	Significance value
BMI, kg/m^2			
1st	23.37±0.48	23.41±0.28	p>0.05
2nd	23.36±0.46	23.79±0.27	p>0.05
3rd	23.36±0.45	23.95±0.28	p>0.05
4th	23.35±0.44	24.20±0.29	p>0.05
LI, ml/kg			
1st	55.75±1.72	55.94±0.63	p>0.05
2nd	57.86±1.69	55.51±0.64	p>0.05
3rd	59.91±1.70	54.97±0.65	p<0.01
4th	61.43±1.71	54.55±0.64	p<0.001
PI, %			
1st	53.57±1.89	53.72±0.91	p>0.05
2nd	56.49±1.86	53.41±0.87	p>0.05
3rd	59.02±1.88	53.24±0.88	p<0.01
4th	63.39±1.90	53.02±0.87	p<0.001
RI, c.u.			
1st	85.34±1.77	85.24±0.67	p>0.05
2nd	83.49±1.76	85.46±0.66	p>0.05
3rd	83.09±1.73	85.62±0.67	p>0.05
4th	82.31±1.68	85.71±0.68	p>0.05
HRR, s			
1st	137.1±3.09	134.8±1.76	p>0.05
2nd	126.3±3.02	129.7±1.73	p>0.05
3rd	117.9±2.97	124.6±1.71	p>0.05
4th	110.4±2.93	119.2±1.70	p<0.05
Physical health level, points			
1st	1.51±0.64	1.47±0.22	p>0.05
2nd	4.98±0.58	1.78±0.21	p<0.001
3rd	7.09±0.60	2.04±0.20	p<0.001
4th	7.21±0.59	2.55±0.21	p<0.001

Legend: Mean – arithmetical average; SD – standard deviation; p – the significance of difference between the indicators of EG and CG due to the Student's t-test

the period of study, the power index improved significantly by 9.82 % in the EG (p<0.001), and did not change authentically in the CG (p>0.05). It indicates a positive effect of sports on strengthening the organisms of future doctors. The estimation of the power index according to Table I showed that the power index of the CG students corresponded to a low level at all stages of the study, and the index of the EG students – to a low level in the 1st – 3rd terms, and below the middle in the 4th term, which emphasizes the advantage of classes in specialized groups in strength sports at the university.

The analysis of the Robinson's index showed that a significant difference between the EG and CG was not detected at all stages of the study (p>0.05). However, during the study, the Robinson's index improved by 3.03 c.u. in the EG, and remained unchanged in the CG (Table IV). It proves a positive impact of sports on improving the functional abilities of the cardiovascular system of students. The evaluation of the Robinson's index defined that the functional abilities reserves of the cardiovascular system of the students of both groups corresponded to the middle level in the 1st term. In all other terms, the Robinson's index was estimated as the middle in the CG, and as above the middle in the EG.

The analysis of heart rate recovery showed that in the 1st – 3rd terms, the indicators of the EG and CG were significantly equal (p>0.05). In the 4th term, the heart rate recovery of the EG students was found to be better than the CG students by 8.8 s (p<0.05) (Table IV). The analysis of the dynamics of the cardiovascular system recovery showed that the heart rate recovery improved in both groups, but it accounted for 15.6 s in the CG (p<0.001), and 26.7 s in the EG (p<0.001). It emphasizes the efficiency of sports in

terms of the improvement of the students' cardiovascular system. The assessment of this parameter in accordance with Table I determined that the heart rate recovery of the CG students corresponded to below the middle level in the 1st – 3rd terms, and the middle in the 4th term; in the EG the below the middle level was recorded in the 1st and 2nd terms, the middle level in the 3rd and 4th terms.

The study of the physical health level showed that the level of health of the EG and CG students was the same authentically only in the 1st term ($p>0.05$). At all other stages of the study, the EG students were recorded to have a significantly higher level of physical health than the CG students ($p<0.001$) (Table IV). The analysis of the dynamics of physical health showed that there was a tendency for health improvement in both groups. However, the level of health improved by 1/08 points in the CG ($p<0.01$), and by 5.7 points in the EG ($p<0.001$) that confirms the results of many studies [5, 13, 17] on the positive impact of sports on improving students' health. The assessment of the physical health level of students in accordance with Table I designated that the level of health corresponded to a low level in the CG at all stages of the study, and in EG, it corresponded to a low level in the 1st term, below the middle in the 2nd and 3rd terms, and the middle in the 4th term.

Thus, the research concludes that strength sports affect physical development, functional abilities of the major systems of an organism, and the health state of future doctors in a more efficient way than the current program of physical education at a medical university. In addition to the development of physical qualities, the morphofunctional development of students and health improvement, physical exercises help increase the body's resistance to adverse factors of educational activities, prevent somatic diseases, prolong active life, increase academic success and professional efficiency of future doctors.

DISCUSSION

The most important human value is health. Good health and high resistance to adverse environmental factors are the important conditions for active longevity, successful learning, productive professional activities, personal and family happiness. Only a physically, spiritually, and mentally healthy person can realize the potential most effectively and at the same time feel like a sound member of society [6, 8, 9].

The studies of many scientists [21, 27] showed that in the developed countries, the issues of physical education and mass sports among students are of great importance, considering them as the most cost-effective and efficient means of preventing diseases, strengthening the gene pool and solving a number of other social problems. Thus, analyzing the experience of the physical improvement process organization at HEI in Poland, Yu. Voyner [28] found that 120 hours are devoted to physical education classes at most of the state HEI (41 % of universities), which are implemented during the 1st and 2nd terms of study. About 28 % of Polish HEIs devote 180 hours to solve the tasks of purposeful physical improvement of students, realized during the 1st – 3rd terms of study. As a rule, after completing the compulsory physical education program of the 1st and 2nd years of study, a student continues to attend elective classes in sports clubs. Moreover, there are significant discounts for tuition or increased scholarships for the active members of sports clubs. In the US, the physical education system of students functions mainly by means of sports training in the relevant sports clubs, so a future specialist has the opportunity to develop physical abilities in the chosen sport, which one considers the most interesting and favorable for physical improvement [29]. In China, the initial courses of study necessarily provide for the solution of general physical training tasks, and in the senior courses, physical improvement is carried out by means of sports training [30].

The main purpose of the physical education of the students of Ukrainian HEI is to increase the physical culture of young people, which primarily involves their engagement in an active lifestyle. Currently, the experts have no doubt that the formation of the students' positive motivation for sports directly affects the efficiency of the physical education process [31, 32]. The conducted research showed that the current system of physical education at HEI of Ukraine is not effective enough to strengthen the health of students. Our results are confirmed by the conclusions of many scientists [1, 4, 12] who believe that the traditional form of physical education classes leads to a decrease in students' interest in physical education. Many scientists [7, 16, 20] argue that one of the most relevant forms of physical education organization is a sports-oriented form of training. Each student can choose the sport that is the most suitable and that brings pleasure. It is necessary to take into account the popularity of sports among students, which may be determined by questionnaires, the opportunities, and conditions of sports facilities of a HEI, as well as sports specialists among the teaching staff of the Department of Physical Education.

The investigation of research, aimed at solving the problems of physical education of students [20, 21, 22], showed that the organization of physical education of students on the basis of their division into groups by interests in sports increases motivation for classes, affects the regularity of their attendance, promotes their health, and increases their physical fitness.

CONCLUSIONS

The conducted research suggests that strength sports in specialized groups of universities are more effective than the current program of physical education for the physical development, functional abilities of the body's major systems, and the level of physical health of future doctors. It was found that the influence on the indicators of handgrip test, vital capacity, and heart rate of the EG students was the most prominent positive effect of sports. The evaluation of the calculated indexes (power index, life index) and the level

of physical health confirmed this trend. It was found that the level of physical health, which was determined by the method of Professor Apanasenko, of the EG students was significantly better than that of the CG students' at the end of the study ($p<0.001$), and was assessed as the middle in the EG, and as a low in the CG. This asserts the necessity of introducing the sports-oriented form of the physical education organization at the medical HEI of Ukraine to maintain the efficiency of the future doctors' professional activity.

The prospects for future research are aimed at studying the level of physical health of university students – future doctors, who in the process of studying attended sports groups in various sports.

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ORIGINAL ARTICLE

PECULIARITIES OF BONE MINERAL DENSITY IN WOMEN OF DIFFERENT REPRODUCTIVE AGE WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT

The aim: To study the peculiarities of bone mineral density in the Ukrainian population of women of different reproductive age with systemic lupus erythematosus and to evaluate its connection with traditional and specific (typical for systemic lupus erythematosus) risk factors.

Materials and methods: A total of 91 women with systemic lupus erythematosus and 29 healthy individuals were examined. Along with the clinical study of the activity and severity of the disease, the serum levels of interleukin-6 were determined by the enzyme immunoassay. The peculiarities of bone mineral density were studied using dual-energy X-ray absorptiometry. The presence of fractures was evaluated by the X-ray method.

Results: Patients with systemic lupus erythematosus frequently suffer from reduced bone mineral density. Reduced bone mineral density and the appearance of fragility fractures are associated with patients' age, disease duration, damage index, inflammatory activity, and cumulative dose of glucocorticoids.

Conclusions: Progressive reduced bone mineral density in patients with systemic lupus erythematosus occurs not only during the aging process of a woman, but is also associated with a number of systemic lupus erythematosus – related osteoporosis risk factors.

KEY WORDS: osteoporosis, fragility fractures, glucocorticoids, interleukin-6

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INTRODUCTION

Nowadays, the worldwide interest of scientists to osteoporosis is primarily motivated by the high prevalence of both the disease itself and its consequences – limb and spine fractures, which cause temporary and permanent disability, poor quality of life and increased mortality [1].

Recent studies have shown a high incidence of osteoporosis and fragility fractures in patients with systemic lupus erythematosus (SLE) compared with the general population [2, 3].

The development of osteoporosis in patients with SLE is thought to be mainly associated with the use of glucocorticoids (GCs) and hyperproduction of cytokines, which negatively affect bone metabolism [4, 5]. The significance of the above mentioned factors is proved by the fact that the reduced bone mineral density (BMD) in the lumbar spine and hip in SLE patients who received large doses of prednisone and suffered from active inflammation is significantly higher than in those with low-grade or moderate-grade inflammation and/or low-dose use of GCs [**5, 6].** Disorders of bone metabolism, development of osteoporosis and its complications in SLE patients are caused not only by disease-specific risk factors, but also by traditional ones, such as sex, motor activity, lifetime, diet, which alone or in combination affect the patients' health. [2]. There are few comprehensive studies of the role of traditional and SLE-specific risk factors for the development of BMD disorders in the world literature, and practically none in Ukraine.

THE AIM

Taking into consideration all mentioned above, the aim of this study is to investigate the bone mineral density in Ukrainian women of different reproductive age with systemic lupus erythematosus and to evaluate its connection with traditional and specific (typical for SLE) risk factors.

MATERIALS AND METHODS

91 women with SLE (the main group) participated in the study. The average age of patients was 45.11 ± 1.03 years. The control group consisted of 29 healthy individuals of the same age and sex (average age – 46.79 ± 2.30 years). The diagnosis of SLE was established on the basis of 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus

Table I. BMD in SLE women of different reproductive age and control group

	Indicator	Characteristic	Control group	SLE patients
		Reproductive period		
1	Z-score ≤ - 2.0 SD	Lumbar spine, n=13/53	0 (0%)	5 (9.4%)*
		Hip, n=13/32	0 (0%)	3 (9.4%)*
2	Z-score > - 2.0 SD	Lumbar spine, n = 13/53	13 (100%)	46 (90.6%)*
		Hip, n = 13/32	13 (100%)	29 (90.6%)*
3	Z-score, average, SD	Lumbar spine, n = 13/53	-0.10 ± 0.15	-0.65 ± 0.14*
		Hip, n =13/32	-0.07 ± 0.13	-0.59 ± 0.16*
4	BMD, g/cm2	Lumbar spine, n = 13/53	0.94 ± 0.04	0.92 ± 0.02
		Hip, n =13/32	1.00 ± 0.05	0.92 ± 0.03
		Postmenopause		
5	Osteoporosis, T-score at or below -2.5 SD	Lumbar spine, n=16/38	1 (6,3%)	7 (18,4%)
		Hip, n=16/22	1 (6,3%)	3 (13,6%)
6	Osteopenia, T-score between -1.0 and -2.5 SD	Lumbar spine, n= 16/38	4 (25.0%)	19 (50.0%)*
		Hip, n = 16/22	4 (25.0%)	10 (45.4%)
7	Normal, T-score at -1.0 SD and above	Lumbar spine, n= 16/38	8 (50.0%)	12 (31.5%)
		Hip, n = 16/22	10 (62.5%)	9 (40.9%)
8	T-score, average, SD	Lumbar spine, n = 16/38	-1.02 ± 0.17	-1.29 ± 0.18
		Hip, n =16/22	-0.82 ± 0.14	-1.0 ± 0.2
9	BMD, g/cm2	Lumbar spine, n = 16/38	0.99 ± 0.03	0.88 ± 0.02*
		Hip, n =16/22	1.01 ± 0.03	0.93 ± 0.03
10	Total number of people with decreased BMD, n=29/91		5 (17.2%)	32 (35.2%)*
11	Average BMD, g/cm2	Lumbar spine, n = 29/91	0.97 ± 0.02	0.90 ± 0.01*
		Hip, n =29/54	1.02 ± 0.03	0.93 ± 0.02*
12	Total number of people with fractures	Lumbar spine, n = 29/91	1 (3.4%)	9 (9.9%)
		Hip, n =29/91	2 (6.9%)	4 (4.4%)

Note: * - significant ($p < 0.05$) differences regarding control group.

Table II. BMD of lumbar spine and hip in SLE patients depending on their age

	Groups of patients Indicator		Age groups, years		
			22-35	36-55	> 55
1	Middle age, years		29.1 ± 0.92	46.5 ± 0.68*	59.2 ± 0.71*
2	Number of surveyed in the group, n		16	63	12
3	Total number of people with decreased BMD		0 (0%)	23 (36.5%)*	9 (75.0%)*
4	Total number of people with fractures		0 (0%)	8 (12.7%)*	5 (41.7%)*
5	Lumbar spine	T-score, SD	-0.58 ± 0.19	-1.20 ± 0.14*	-1.31 ± 0.42
		BMD, g/cm2	0.98 ± 0.02	0.89 ± 0.02*	0.88 ± 0.05
		Z-score, SD	-0.62 ± 0.15	-0.68±0.14	-0.53 ± 0.50
6	Number of surveyed in the group, n		9	37	8
7	Hip	T-score, SD	-0.11 ± 0.25	-1.08 ± 0.16*	-1.13 ± 0.31*
		BMD, g/cm2	1.01 ± 0.04	0.92 ± 0.02*	0.90 ± 0.06
		Z-score, SD	-0.08 ± 0.22	-0.79 ± 0.16*	-0.70 ± 0.35

Note: * - significant ($p < 0.05$) differences regarding patients of the smallest age group.

erythematosus [7]. The activity of SLE was evaluated by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [8]. Internal organs damage was determined using Systemic Lupus International Collaborating Clinics/ American College of Rheumatology damage index (SLICC/ ACR DI) [9]. All patients received glucocorticoids and

Table III. BMD in SLE patients depending on the duration of the disease

	Indicator		Duration of the disease, years		
			< 6	6-10	> 10
1	Duration of the disease, years		2.7 ± 0.39	7.9 ± 0.30*	19.0 ± 1.07*
2	Number of surveyed in the group, n		23	16	52
3	Total number of people with decreased BMD		2 (8.7%)	4 (25.0%)	26 (50.0%)*
4	Total number of people with fractures		2 (8.7%)	2 (12.5%)	9 (17.3%)
5	Lumbar spine	T-score, SD	-0.57 ± 0.19	-1.26 ± 0.24*	-1.29 ± 0.17*
		BMD, g/cm2	0.97 ± 0.02	0.89 ± 0.03*	0.88 ± 0.02*
		Z-score, SD	-0.35 ± 0.20	-0.73 ± 0.19	-0.64 ± 0.17
6	Number of surveyed in the group, n		8	9	37
7	Hip	T-score, SD	-0.19 ± 0.38	-0.73 ± 0.31	-1.11 ± 0.15*
		BMD, g/cm2	1.03 ± 0.07	0.92 ± 0.05	0.91 ± 0.02
		Z-score, SD	-0..6 ± 0.32	-0.51 ± 0.35	-0.80 ± 0.16

Note: * - significant differences ($p < 0.05$) regarding patients with disease duration up to 6 years.

Table IV. BMD of the lumbar spine and hip in SLE patients depending on the cumulative dose of glucocorticoids

	Groups of patients Indicator		Cumulative dose of glucocorticoids, g		
			< 19.0	19.0-70.7	> 70.7
1	Average cumulative dose of glucocorticoids, g		9.23 ± 1.19	43.0 ± 2.43*	93.1 ± 5.97*
2	Number of surveyed in the group, n		24	44	23
3	Number of people with reduced BMD		1 (4.1%)	20 (45,4%)*	11 (47.8%)*
4	Number of people with fractures		1 (4.2%)	8 (18,2%)*	4 (17.4%)
5	Lumbar spine	T-score, SD	-0.48 ± 0.25	-1.23 ± 0.11	-1.63 ± 0.22*
		BMD, g/cm2	0.98 ± 0.03	0.89 ± 0.01*	0.84±0.02*
		Z-score, SD	-0.11 ± 0.28	-0.72 ± 0.11	-0.98 ± 0.21*
6	Number of surveyed in the group, n		8	31	15
7	Hip	T-score, SD	-0.43 ± 0.33	-0.82 ± 0.15	-1.37 ± 0.25*
		BMD, g/cm2	0.99 ± 0.04	0.94 ± 0.03	0.87 ± 0.03*
		Z-score, SD	-0.46 ± 0.31	-0.70 ± 0.17	-0.88 ± 0.27

Note: * – significant differences ($p < 0.05$) regarding patients with cumulative dose of glucocorticoids up to 19 g.

did not use other immunosuppressive agents. Partvicular attention was paid to the study of musculoskeletal system and the presence of traditional and SLE-related risk factors for osteoporosis: age, menstrual status, duration of the disease, activity of the inflammatory process, cumulative dose of glucocorticoids, fragility fractures.

The laboratory examination included determination of serum level of interleukin-6 (IL-6) by enzyme immunoassay.

The presence of vertebral fractures was assessed by Genant semi-quantitative method, which requires a 20% decrease in vertebral height on lateral thoracolumbar radiographs. Patients who had a history of femoral neck fractures had X-ray of both hip.

Changes in BMD of the lumbar spine at the level of L1-L4 and the proximal femur were determined by dual-energy X-ray densitometry (DXA) on Hologic Discovery Wi (S / N 87227). The results of BMD determination were expressed in absolute values as well as T- and Z-score. Osteoporosis was diagnosed if the T-score in postmenopausal women of the lumbar vertebrae (L1-L4) and/or proximal femur was -2.5 SD (standard deviation) or less. Osteopenia met T-score from -1.0 to -2.5 SD. In women of reproductive age, the Z-score was used to determine BMD. Values of the Z-score ≤ -2.0 SD were considered as "below the expected range for age". The group of patients with reduced BMD included women of reproductive age with Z-score ≤ -2.0 SD and post-menopausal women with T-score < -1.0 SD.

The study was carried out in compliance with the provisions of the Council of Europe Convention on Human Rights and Biomedicine, Declaration of Helsinki and recommendations of the Committee on Bioethics of the Presidium of National Academy of Medical Sciences of Ukraine.

Statistical analysis of the results was carried out using standard methods with Excel 10.0 application package and using the program "SPSS-10.0.5 for Windows" (license number 305147890). Average value (M) and standard errors (m) were evaluated. The normality of the distribution

Table V. BMD of lumbar spine and hip in SLE patients depending on DI and SLEDAI

	Groups of patients		DI, points		SLEDAI, points	
			< 4	≥ 4	< 20	≥ 20
1	Number of surveyed, n		52	39	67	24
2	Number of people with reduced BMD		10 (19.2%)	22 (56.4%)*	20 (29.9%)	12 (50.0%)*
3	Number of people with fractures		4 (7.7%)	9 (23.1%)*	4 (6.0%)	9 (37.5%)*
4	Lumbar spine	T-score, SD	-0.86 ± 0.14	-1.41 ± 0.19*	-0.94 ± 0.13	-1.51 ± 0.23*
		BMD, g/cm2	0.94 ± 0.02	0.86 ± 0.02*	0.92 ± 0.01	0.86 ± 0.03*
		Z-score, SD	-0.44 ± 0.13	-0.76 ± 0.21	-0.50 ± 0.13	-0.77 ± 0.24
5	Number of surveyed, n		26	28	43	11
6	Hip	T-score, SD	-0.55 ± 0.18	-1.14 ± 0.23*	-0.73 ± 0.12	-1.56 ± 0.36*
		BMD, g/cm2	0.96 ± 0.03	0.90 ± 0.03	0.95 ± 0.02	0.84 ± 0.04*
		Z-score, SD	-0.50 ± 0.17	-0.84 ± 0.20	-0.57 ± 0.14	-1.11 ± 0.31

Note: * - significant ($p < 0.05$) differences regarding patients with low DI (< 4 points) and low SLEDAI (< 20 points).

Table VI. BMD of lumbar spine and hip in SLE patients depending on serum IL-6

	Groups of patients Indicator		IL-6		
			Optimal IL-6 level (< 12.5 ng/L)	Extremely high IL-6 level (12.5-20.0 ng/L)	High IL-6 level (> 20.0 ng/L)
1	Number of surveyed in group, n		24	39	21
2	Number of people with reduced BMD		5 (20.8%)	15 (38.5%)	11 (52.3%)*
3	Number of people with fractures		2 (8.3%)	5 (12.8%)	6 (28.6%)*
4	Lumbar spine	T-score, SD	-0.42 ± 0.26	-1.16 ± 0.12*	-1.97 ± 0.19*
		BMD, g/cm2	0.98 ± 0.03	0.90 ± 0.01*	0.81 ± 0.02*
		Z-score, SD	0.13 ± 0.30	-0.61 ± 0.11*	-1.38 ± 0.20*
5	Number of surveyed in group, n		16	25	13
6	Hip	T-score, SD	0.17 ± 0.31	-1.0 ± 0.18*	-0.96 ± 0.25*
		BMD, g/cm2	1.01 ± 0.03	0.93 ± 0.03*	0.82 ± 0.03*
		Z-score, SD	-0.09 ± 0.17	-0.70 ± 0.17*	-1.36 ± 0.19*

Note: * – significant differences ($p < 0.05$) regarding patients with optimal IL-6 levels.

of indicators was determined by the Shapiro-Wilk test. In our studies, there was a normal distribution of indicators, so the significance of the differences between groups was determined by the Student's t-test. To compare the significance of the differences between the relative values, Fisher's exact test was used. $P < 0.05$ was considered to be significant difference.

RESULTS

9.4% of patients of reproductive age with SLE had reduced BMD in the lumbar spine and hip (Table I). During this period, no cases of bone loss were found in healthy individuals.

In postmenopausal women, osteoporosis in the lumbar spine was observed in 7 (18.4%) patients with SLE, in the hip – in 3 (13.6%). In the control group, osteoporosis was detected in 1 (6.3%) person. Osteopenia occurred in almost every second patient with SLE and in 4 (25.0%) people from the control group. Thus, the total proportion of people with reduced BMD among SLE patients was 32 (35.2%) and 5 (17.2%) among healthy individuals.

The BMD of both study areas in patients with SLE was also lower than in healthy individuals. Thus, the average lumbar spine BMD at reproductive age did not differ significantly in the control and main groups, and the hip BMD was 10% lower in patients with SLE. In postmenopausal women, the lumbar spine and hip BMD in healthy individuals was 0.99. ± 0.03 g/cm^2 and 1.01 ± 0.03 g/cm^2 and was almost 10% higher than in patients with SLE.

Overall, the lumbar spine BMD in individuals from the main group was 0.90 ± 0.01 g/cm^2, at the hip BMD – 0.93 ± 0.02 g/cm^2. In the control group, it was 0.97 ± 0.02 g/cm^2 and 1.02 ± 0.03 g/cm^2, respectively, and was on average 8.0% higher.

As for fragility fractures, they were detected in 13 (14.2%) patients with SLE. In particular, 4 (4.4%) had femoral neck fractures, and 9 (9.9%) had vertebral body fractures. In the control group, fractures occurred in 3 (10.3%) people, 1 (3.4%) woman had a vertebral fracture and 2 (6.9%) women had femoral neck fractures.

The study found that one of the factors that negatively affect the bone tissue is the age of patients (Table II). In particular, there were no patients with reduced BMD and fractures in the age group of 22-35 years. The proportion of people with reduced BMD and fractures in the 36-55-year group was 36.5% and 12.7%, respectively. Among patients over 55 years of age, reduced BMD was observed in 75.0% of the examined patients, i.e more than twice as often as in 36-55-year-old individuals. During this period, the incidence of fractures was 41.7%. BMD and average Z- and T-score in the lumbar spine and proximal femur also decreased in proportion to the age of the woman.

Bone loss in SLE patients was closely associated with disease duration (Table III).

These associations were more prominent in the lumbar spine. Thus, in people with disease duration up to 6 years BMD was equal to 0.97 ± 0.02 g/cm^2, while in persons with disease duration of 6-10 years it was 0.89 ± 0.03 g/cm^2, and in patients with disease duration more than 10 years it was 0.88 ± 0.02 g/cm ^{2} or it was 9.2% lower. This was also confirmed by the increase in the proportion of people with reduced BMD among patients with moderate and high disease duration. In the latter two groups in particular, it was 25.0% and 50.0%, respectively. The incidence of fractures was also higher in women with the highest disease duration (> 10 years).

An increase in the cumulative dose of glucocorticoids was found to have a negative effect on BMD in patients with SLE (Table IV). Thus, the lumbar spine BMD in patients with relatively low dose of glucocorticoids was significantly higher (9.2% and 14.2%, respectively) than in patients with high and very high cumulative dose of glucocorticoids. Similar changes were found in the hip. In both cases, an increase in the number of patients with reduced BMD and fragility fractures was observed.

The results of studies have shown that reduced BMD in women with SLE is closely related to the course of the disease – SLEDAI and the damage index (Table V). In particular, in persons with low disease activity (SLEDAI < 20 points), BMD in both study areas was on average 9.0% higher than in persons with high disease activity (SLEDAI ≥ 20 points). In patients with high damage index (DI ≥ 4 points), the lumbar spine BMD was also significantly (8.5%) lower than in women with DI < 4 points. The T- and Z-score also decreased in proportion to the increase of inflammation and an increase in the damage index.

In women with SLE, increased serum IL-6 level also negatively affected the bone tissue (Table VI). Thus, in persons with high inflammation activity the lumbar spine BMD was 17.3% (the hip BMD – 18.8%) lower than that at the optimal level of IL-6 and was equal to 0.81 ± 0.02 g/cm^2 (0.82 ± 0.03 g/cm ^{2}) against 0.98 ± 0.03 g/cm^2 (1.01 ± 0.03 g/cm^2). Simultaneously with the increase in serum IL-6, the proportion of individuals with reduced BMD increased. Analysis of the number of fractures in patients with SLE showed the highest value of this indicator in women, with high values of IL-6.

DISCUSSION

The study showed that BMD decrease was observed among 35.2% of Ukrainian women with SLE and 17.2% of the control group. In particular, 11.3% of premenopausal patients had a Z-score below the expected range for age. In the postmenopausal period, osteopenia was detected in 50.0% of women with SLE, and osteoporosis – in 18.4%. As for the healthy persons, there were no premenopausal women with BMD below the expected range for age. In the postmenopausal control group, the prevalence of osteoporosis and osteopenia was 6.3% and 25.0%, correspondingly.

According to the research of Cramarossa G. et al. (2017), who, like us, have studied bone mineral density in women with SLE of different reproductive age using T- and Z-score and found that premenopausal women have reduced BMD in 17.3% of individuals. In postmenopausal women, osteopenia was detected in 43.2% of cases, and osteoporosis – in 12.3% [10].

Other data also indicate a high incidence of osteoporosis and osteopenia in patients with SLE. For example, osteoporosis was found in 1.4% and even in 68.0% of people, osteopenia – 25.0-74.0% of cases [2].

Bone mineralization evaluated by BMD was also significantly lower in patients with SLE. Thus, in the lumbar spine in the main study group, it averaged 0.90 ± 0.01 g/cm^2, while in healthy individuals, 0.97 ± 0.02 g/cm^2. The hip BMD in patients with SLE was 0.93 ± 0.02 g/cm^2, and in the control group it was 1.02 ± 0.03 g/cm^2. Our results fully coincide with the literature data. Wang et al. conducted a meta-analysis of 15 researches devoted to the study of the condition of bone tissue in patients with SLE. They report that patients with SLE suffer from an absolute reduction in BMD (an average of 0.06 g/cm^2), detected in the lumbar spine and femoral neck compared with the control group [11].

We have shown that in 14.2% of patients with SLE, fragility fractures occurred. The obtained data regarding the high prevalence of fractures in patients with SLE are consistent with the results of a number of studies. Thus, recent studies have demonstrated that high frequency (13.7-50%) vertebral fractures are reported in SLE patients of both sexes, with every third patient having normal BMD [4, 12-15].

One of the adverse factors affecting the condition of bone tissue is the age of patients. According to the literature, older age is closely associated with reduced BMD in patients with SLE [10]. Similar patterns were found in our study. An analysis of the incidence of fractures showed that it was significantly higher among older SLE patients than among the youngest ones.

The negative effect of the duration of the disease on the condition of bone mineral density was established. In particular, T-score and the lumbar spine BMD progressively decreased with increasing time from the onset of the disease. Similar results were obtained in other studies [10, 16].

It is known that long-term use of glucocorticoids is a significant factor in determining the condition of bone tissue in patients with rheumatic diseases [17]. Recent studies have clearly indicated that there is no safe dose of glucocorticoids

for bone tissue [18]. Our analysis of the relationship between BMD and glucocorticoids load revealed that the process of bone loss and, which results in fragility fractures, is associated with an increase in the cumulative dose of GC. In particular, BMD of both study areas at the cumulative dose of GC > 70.7 g was, on average, 13.2% lower than that of a relatively low GC. In addition, the proportion of patients with low BMD also increased significantly in proportion to the increase in cumulative dose of GCs. The obtained results confirm the data of other researchers [10, 19].

Another pathogenic factor of the adverse effect on bone tissue in patients with SLE is a systemic inflammatory process. An excess of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) induces osteoclastogenesis by enhancing osteoclast differentiation, contributing to increased RANKL synthesis and individual prostaglandins having resorptive activity [20]. However, the current views on this issue are quite contradictory. According to Pineau CA et al. (2004), García-Carrasco M et al. (2009), Cramarossa G et al. (2017), Sun YN et al. (2015) reduced BMD in patients with SLE was not associated with the inflammatory activity, while the overwhelming majority of scientists concluded that one of the predictors of osteoporosis is the damage index [10, 21-26] and high disease activity [14, 27, 28].

Our research has shown that reduced BMD in women with SLE is associated with an increase in IL-6 and damage index. In particular, individuals with high inflammatory activity, the lumbar spine BMD was 17.3% lower (18.8% – the hip BMD) than those with the optimal IL-6 level. The presence of fractures in women with SLE showed a very close dependence on the damage index, SLEDAI, as well as the level of IL-6. Thus, among individuals with high levels of IL-6, the proportion of individuals with fractures was 3.4 times higher than in the group with the optimal level of cytokine.

Thus, in patients with SLE there is a high frequency violation of bone tissue. The progressive decrease in BMD, which occurs not only during the aging process of a woman, is associated with a number of osteoporosis risk factors (duration of disease, high levels of inflammatory markers, use of glucocorticoids).

CONCLUSIONS

1. In 35.2% of women with SLE, reduced bone mineral density was observed. In particular, 11.3% of patients of reproductive age had Z-score below the expected range for age. In the postmenopausal period, osteopenia was found in 50% of women, osteoporosis – in 18.4%. Reduced bone mineral density is associated not only with the age of patients, but also with the duration of the disease, damage index, the inflammatory activity and the cumulative dose of glucocorticoids.
2. Fragility fractures in women with SLE are found in 13 (14.2%) persons, of which femoral neck fractures in 4 (30.7%), vertebral body fractures in 9 (69.3%). The presence of bone fractures has no association with the duration of the disease, but is also associated with the dose of GCs, disease course (IL-6 level, damage index) and age of patients.

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ORIGINAL ARTICLE

COMPLEX THERAPY OF ATOPIC CHEILITIS

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ABSTRACT

The aim: Was to evaluate clinical data after the use of pimecrolimus (1% cream "Elidel") in patients with mild and moderate severity of atopic cheilitis, according to modern therapeutic requirements. There was an algorithm of treatment the patients with cheilitis proposed it based on the data from literary sources and personal clinical experience.

Materials and methods: 22 patients with atopic cheilitis aged from 19 to 40 agreed on a clinical examination in accordance with the protocol of the study. Patients were prescribed "Elidel" in the form of 1% cream for lubrication of the affected areas of the skin and lips, and antihistamine "Erius", for the normalization of the general condition used sedatives and vitamins after consultation of specialists in the general profile.

Results: Patients of both groups during the re-examination after 3, 7, 10 days recorded a positive dynamics of the red border of the lips and skin: a significant reduction in the inflammatory process, normalization of indicators of general blood analysis, improvement in the overall quality of life of patients.

Conclusions: The results of treatment allow to consider 1% cream "Elidel" (pimecrolimus) as a preparation of choice in the complex treatment of patients with atopic cheilitis of mild and moderate severity.

KEY WORDS: atopic cheilitis, treatment

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INTRODUCTION

Atopic cheilitis (cheilitis atopicalis) – AC is a chronic inflammatory recurrent disease of the lips. According to literary sources, AC affects up to 25% of children and 2-10% of adults, the incidence rate in the last 30 years has grown 2-3 times in developed countries. The disease is a symptom of atopic dermatitis or neurodermatitis and has a significant influence on the quality of life of both patients and members of their families [1,2].

The pathogenesis of this disease is still not thoroughly studied, but there is an opinion that the disease occurs as a result of a complex interaction between the violation of the barrier function of the mucous membrane and skin, immune dysfunction and environmental factors or agents of infectious origin. The break of the epithelial barrier leads to the loss of transepithelial fluid, increased sensitivity to infectious and allergic agents. In most cases, AC is characterized by IgE, hypersecretion and violation of cytokine regulation in the ratio of Th1 / Th 2 –lymphocytes, determined deficiency of T-lymphocyte suppressors, disruption of the processes of apoptosis. Clinical symptomatology is caused by allergic inflammation with infiltration by T-lymphocytes and eosinophils. The hyperactive condition of the immune cellular chain in the cascade of interaction between inflammatory infiltrate cells, blood vessels and keratocytes is a key factor in the development of the disease and its clinical manifestations [3, 4].

Since AC is a chronic recurrent disease, its clinical management requires comprehensive, long-term therapy. The task is to reduce symptoms, to restore the broken epithelial barrier and to enhance the quality of life. In addition, treatment should be aimed at the regulation of immune dysfunction, preventing progression of the disease and prolonging the remission period [4].

As a rule, the basic standard AC therapy includes: facial skin and lips care, elimination contacting factors that trigger aggravation; maintenance therapy in the form of mitigating agents that restore barrier function of the skin and mucous membrane; anti-inflammatory therapy by the use of topical corticosteroids (TCs) and topical calcineurin inhibitors (TCI) [5,6].

For a long time (more than 20 years), in our practice we used TCI – hydrocortisone acetate (1%) or hydrocortisone butyrate (0.1%) as applications for the affected skin of the face and red border of the lips [2].

Now, according to the clinical guidelines of the state expert center (2016) at the Ministry of Health of Ukraine, we use topical calcineurin inhibitors (TCI). It is a non-steroidal immune modulator – tacrolimus ("Protopic") in the form of 0.03% and 0.1% ointment and pimecrolimus ("Elidel") in the form of 1% cream. TICs have a more specific mechanism of action, they selectively suppress the activation of T-cells, mast cells, and the formation of anti-inflammatory cytokines.

THE AIM

The aim of our study was to evaluate clinical data after the use of pimecrolimus (1% cream "Elidel") in patients with mild and moderate severity of atopic cheilitis, according to

modern therapeutic requirements. There was an algorithm of treatment the patients with cheilitis proposed it based on the data from literary sources and personal clinical experience.

MATERIALS AND METHODS

22 patients with atopic cheilitis aged from 19 to 40 agreed on a clinical examination in accordance with the protocol of the study. 13 patients were diagnosed with atopic cheliitis of light severity and isolated lesion of lips was observed. Anamnesis of these patients' states that they do not visit doctor regularly due to this pathology. At first, according to the prescription and later independently apply corticosteroid ointments until the disappearance or reduction of symptoms of the disease. In nine patients from 2nd group lesions of the lips, skin of the upper limbs and eyelids were detected. They noticed itching, peeling of the lips and damaged skin. They are treated continuously, and, as a rule, using hormones in the dosage form of ointments. The red border in all patients was congested hyperemic, squamous, lichenization and infiltration of the skin in the corners of the mouth were noted. General blood analysis stated lymphocytosis and eosinophilia in all patients. Concomitant pathology was noted in 63.5% of patients, prevalence of pathology of the gastrointestinal tract, vasomotor dyscrasia and allergic conditions. 82% of patients with atopic cheilitis complained of life quality deterioration, to be exact, that the disease affects daily life, learning, social communication. Patients are anxious, depressed due to skin and lips lesions. Intensive itching, which lasts throughout the day and intensifies at night, leads to insomnia in 40.9% of patients.

Patients in both groups were prescribed "Elidel" in the form of 1% cream for lubricating the affected areas of the skin and lips, and as an antihistamine, a long-acting third-generation drug "Erius" in the form of tablets for intraoral administration. In which active substance is desloratadine with triple (antihistamine, anti-allergic and anti-inflammatory) effect. This choice was not accidental. According to the manufacturer, "Erius" suppresses the cascade of various reactions that underlie allergic inflammation, such as: the secretion of proinflammatory cytokines, including IL-4, IL-6, IL-8, IL-13; the secretion of proinflammatory chemokines such as RANTES; production of superoxide anion activated polymorphonuclear neutrophils; adhesion and chemotaxis of eosinophils; IgE-dependent release of histamine, prostaglandin D2 and leukotriene C4.

In addition, to normalize the general condition, sedative and vitamin preparations were used after consultation by general practitioners.

The studies were carried out in compliance with the main provisions of the "Rules of Ethical Principles for Conducting Scientific Medical Research with Human Participation", approved by the Declaration of Helsinki (1964-2013), ICH GCP (1996), EEC Directive No. 609 (dated 24.11.1986), orders of the Ministry of Health of Ukraine No. 690 dated September 23, 2009, No. 944 dated December 14, 2009, No. 616 dated August 3, 2012. For participation in the study, patients signed the form of "Voluntary informed consent of the patient to participate in the study".

RESULTS AND DISCUSSION

Patients in both groups during repeated inspection after 3, 7, and 10 days showed a positive dynamics of normalization of the condition of the red border of the lips and skin. Patients of the 1st group noticed an improvement after 3 days of the usage of 1% of the "Elidel" cream for a number of indicators – redness, itching and partial peeling of the red border of the lips disappeared.

Patients of the 2nd group on the 3rd day of treatment itching and redness disappeared, on the 7th day, infiltration and peeling of the red border of the lips disappeared, on the 10th, the phenomena of lichenization significantly decreased.

In both groups, positive dynamics was noted in the general blood test, a significant decrease in the inflammatory process clinics and a significant improvement in the quality of life of the patients.

The obtained results made it possible to propose an algorithm for the treatment of patients with atopic cheilitis, which is performed after oral cavity sanitation as part of complex therapy (allergen elimination, diet, use of softening cosmetic products) as follows:

- Carefully clean the skin and red border of lips with antiseptic;
- apply a thin layer of 1% cream of "Elidel" on the affected skin and red border of the lips twice a day until the symptoms disappear completely;
- prescribe as an antihistamines drug "Erius" (5 mg) 1 tablet once a day (the duration of treatment is determined by the course and severity of the disease);
- recommend the use of a softening cream for red border and face skin at night;
- educate patients for skin and lips care (avoid contact with irritants and allergens).

"Elidel" once a day for 3 months on those areas where there were elements of lesions and use of moisturizing and softening creams for the skin, hygienic lipstick for the red border of the lips.

CONCLUSIONS

Thus, atopic cheilitis is a disease requiring complex long-term therapy aimed at regulation of immune dysfunction and consists of the symptoms mitigation, the epithelial barrier restoration, the disease progression prevention, and extension of the remission period.

The results of treatment allow to consider 1% cream "Elidel" (pimecrolimus) as a preparation of choice in the complex treatment of patients with atopic cheilitis of mild and moderate severity.

The proposed algorithm for treatment of atopic cheilitis is easy to implement, it is directed primarily at the elements of pathogenesis, which allows to control the symptoms of

the disease (itching, edema, erythema), prevents the development of complications (transition to the eczematous form), contributes to the restoration of affected areas of the skin and red borders of the lips, and also significantly improves the quality of life of this contingent of patients.

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ORIGINAL ARTICLE

METABOLIC SYNDROME, OVERWEIGHT, HYPERLEPTINEMIA IN CHILDREN AND ADULTS

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ABSTRACT

The aim: To evaluate anthropometric, hemodynamic parameters, as well as changes in blood and leptin lipid spectrum in children and adults with overweight and obesity.

Materials and methods: We examined 68 overweight children and 90 patients with obesity in combination with stage 2, grade 2 AH who were electively inpatient. The control group consisted of practically healthy individuals – 20 adults and 55 children.

Results: Obesity in childhood isaccompanied by the development of dyslipidemia, hypercholesterolemia, hyperleptinemia and hypertension, and in adulthood may be an additional risk factor for cardiovascular disease, in particular AH. According to the study, total leptin level in overweight children was significantly higher compared to the control group ($p<0.01$). The concentration of leptin in patients with hypertension in combination with obesity was 3 times higher compared to the control group ($p<0.01$)

Conclusions: Thus, obesity or overweight, accompanied by hyperleptinemia and an increase in the proatherogenic fractions of the blood lipid spectrum, is an important problem that needs to be addressed in childhood to prevent cardiovascular disease in adulthood.

KEY WORDS: arterial hypertension, obesity, overweight, leptin

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INTRODUCTION

At present, obesity and overweight have become one of the most serious public health problems, due to the significant increase of their prevalence among population of different age groups in all countries [1, 2, 3, 4]. According to prognosis, by 2030, an increase in excess body weight is expected in adults up to 86.3% and obesity up to 51.1% [5].

In the age-related population, overweight occurs in 20% of children and teenagers. It is estimated that almost every 5th European child has abnormal body weight [6, 7]. The highest incidence of overweight in teenagers is 28.9% in boys and 16.4% in girls in Greece. In Italy, almost 36% of children aged 9 are overweight or obese, and in Spain, 26.6% of children aged 9-13 are diagnosed with overweight, almost 4% are obese [6, 7]. In overweight and obese children, hyperleptinemia is found in 80% of cases, in adults with metabolic syndrome (MS) hyperleptinemia is found in 72% [8]. In adults, hyperleptinemia is an independent factor for the development of coronary heart disease and arterial hypertension.

The manifestation of obesity is MS, which contributes to the occurrence of atherosclerosis, type 2 diabetes, which leads to increased mortality in the population [9, 10]. One of the components of MS is abdominal obesity. An important role in the development and progression of metabolic disorders is played by adipose tissue, which synthesizes a large number of adipocytokines, among which hyperleptinemia plays an important role in the occurrence of coronary heart disease, hypertension, diabetes and stroke. [9, 11]. High levels of leptin in the blood plasma are also accompanied by activation of the sympathetic nervous system, endothelial dysfunction, oxidative stress, proinflammatory and prothrombotic disorders [8, 10].

Despite the considerable body of research on the pathogenesis of MS, this issue needs further study in terms of search of effective prevention of obesity-related diseases.

THE AIM

The aim of the study was to evaluate anthropometric, hemodynamic parameters, as well as changes in blood and leptin lipid spectrum in children and adults with overweight and obesity.

MATERIALS AND METHODS

We examined 68 overweight children and 90 patients with obesity in combination with stage 2, grade 2 AH who were electively inpatient. The control group consisted of practically healthy individuals – 20 adults and 55 children.

Among the surveyed children, 49 (72%) girls were overweight, boys – 19 (28%), average age was 13.12±0.38. In the group of patients with combined pathology (AH with obesity), there were 49 (54.4%) men, women – 41 (45.6%), the average age was 57.49±1.07.

All patients underwent anthropometric, general clinical, laboratory (blood lipid spectrum), instrumental – electro-

Table I. Anthropometric and hemodynamic parameters in children and adults with overweight and obesity

Indicators	Control group (n=55)	68 overweight children	Control group (n=20)	90 patients with hypertension and obesity
BMI, kg/m^2	19,10±0,31	28,82±0,49**	23,98±0,16	32,24±0,52**
WV, cm	68,00±1,00	96,60±0,81**	73,80±0,92	95,87±1,33**
TV, cm	85,00±1,29	106,01±1,73**	91,38±0,34	91,92±0,55
Index WV/ TV	0,78±0,01**	0,86,±0,02	0,80±0,01	0,96±0,01*
SBP, mm Hg,	110,00±1,23	133,11±2,05**	132,00±0,82	168,20±2,28*
DBP, mm Hg,	70,01±1,63	80,49±1,50**	76,50±1,07	107,20±0,66*

Note: ** p <0,05 compared to control group, * p <0,01 compared to control group

Table II. Indicators of lipid spectrum of blood and leptin in children and adults with overweight and obesity

Indicators	Control group (n=55)	68 overweight children	Control group (n=20)	90 patients with hypertension and obesity
TC, Mmol/l	3,60±0,15	4,54±0,14*	4,32±0,09	6,05±0,13*
TG, Mmol/l	1,06±0,06	1,35±0,08*	1,47±0,06	2,13±0,13*
LDL, Mmol/l	1,78±0,09	2,44±0,11*	2,62±0,09	3,63±0,05*
HDL, Mmol/l	1,31±0,07	1,42±0,06	1,25±0,04	1,14±0,05*
Leptin, ng/ml	7,88±2,06	27,65±3,67**	10,54±1, 02	31,72±2,59**

Note: ** p <0,05 compared to control group, * p <0,01 compared to control group

cardiography, echocardiography, ultrasonography (USG) and enzyme immunoassay (leptin).

The diagnosis of AH was established using standards of diagnosis and treatment in accordance with the recommendations of the Ukrainian Association of Cardiologists, the European Society of Hypertension and the European Society of Cardiologists (ESH / ESC). Plan of examination and treatment tactics was guided by the order of the Ministry of Health of Ukraine No. 384 of 24.05.2012, «On approval and implementation of medical and technological documents on standardization of medical care for arterial hypertension».

Patients were measured for height and weight, body mass index was calculated according to conventional formulas.

Office blood pressure was measured according to standard of examination. Measured systolic blood pressure (SBP) and diastolic blood pressure (DBP)/ Heart rate was determined after the second pressure measurement.

All persons underwent USG of the internal organs, upon which special attention was paid to the increase in the size of the liver, the density of its parenchyma, diffuse uniform increase in echogenicity, the appearance of the effect of distal shading and the diameter of the portal vein.

All enzyme immunoassay methods were performed using a «Statfax 303 plus» analyzer (AwarenessTechnology, USA).

Leptin content was determined using the DRG «Leptin ELISA» kit (Germany), using on the sandwich principle. Research was conducted according to the method of enzyme immunoassay.

The results were statistically processed using the Student's t test, Pearson correlation analysis using the «MS Excel software».

RESULTS AND DISCUSSION

The study enrolled inpatient patients, who underwent treatment with AH stage II, grade 2 moderate, high and very high risk in combination with obesity and children undergoing outpatient care by a family physician due to overweight.

Overweight was diagnosed in 25 (36.76%) children, respectively.

Obesity of the 1st degree was diagnosed in 61 (67.78%) patients, 2nd – in 22 (24.44%), 3rd degree – in 7 (7.78%). Obesity was observed in 87 (96.67%) patients. Significant increase in total body weight, BMI, thigh volume (TV), and TV / waist volume (WV) index was found in overweight children and in the group of patients with combined pathology compared with the control group ($p<0.05$). There was no significant difference in WV in the group with combined pathology (Table 1).

The mean level of SBP and DBP was significantly higher in overweight children ($p<0.05$) and in patients with combined pathology ($p<0.01$) compared with controls (Table 1).

According to the results of USG, in children with overweight there was a slight increase in the size of the liver 18 (26.47%) and in 34 (50.0%) children we revealed the heterogeneity of its structure and increased echogenicity of the parenchyma.

An ultrasonographic increase of liver size was found in 90 (81.8%) subjects with AH with obesity, such changes were absent in control subjects. Hyperechogenicity of the liver parenchyma was observed in 89 (80.9%) of the examined persons, heterogeneity of the liver structure was diagnosed in 83 (75.4%) persons.

The study of the lipid spectrum of the blood in the majority of patients indicates on significant increase in the levels of proatherogenic fractions, unlike the control group. Dyslipidemic changes were found in 59 (87.1%) overweight children and 86 (95.56%) patients with AH combined with obesity. In particular, an increase in total cholesterol (TC) levels was observed in 86 (95.56%) patients, low density lipoprotein (LDL) in 60 (66.67%), triglycerides (TG) – in 47 (52.22%). The level of high density lipoprotein (HDL) was maintained within the normal range in 45 patients (50.0%) (Table 2).

According to the study, total leptin level in overweight children was significantly higher compared to the control group ($p<0.01$). The concentration of leptin in patients with hypertension in combination with obesity was 3 times higher compared to the control group ($p<0.01$) (Table 2). An increase in leptin levels above the reference values was detected in 80% of the surveyed individuals, indicating on impaired metabolic processes in combined pathology.

It is known that the use of statins, in particular atorvastatin for the correction of blood lipid spectrum helps to reduce leptin levels in adults [12, 13]. However, the prescription of statins in children is impractical because of many side factors. As a result, the problem of preventing obesity in children requires lifestyle adjustments.

Obesity in childhood is accompanied by the development of dyslipidemia, hypercholesterolemia, hyperleptinemia and hypertension, and in adulthood may be an additional risk factor for cardiovascular disease, in particular AH [14, 15].

CONCLUSIONS

Thus, obesity or overweight, accompanied by hyperleptinemia and an increase in the proatherogenic fractions of the blood lipid spectrum, is an important problem that needs to be addressed in childhood to prevent cardiovascular disease in adulthood.

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ORIGINAL ARTICLE

ACTIVITY OF SUPEROXIDE DISMUTASE AND CATALASE IN THE GASTRIC MUCOSA OF RATS UNDER THE PROLONGED ADMINISTRATION OF OMEPRAZOL AND COMBINATION OF OMEPRAZOLE AND MULTIPROBIOTICS

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ABSTRACT

The aim: Of the study was to study the activity of superoxide dismutase and catalase in the gastric mucosa of rats under long-term administration of omeprazole and combined administration of omeprazole with Symbiter and Apibact multiprobiotics.

Materials and methods: The study was carried out on 40 white non-linear male rats with an initial weight of 160-180 g. All animals were divided into 4 groups. Group I was the control. Group II was administered omeprazole once a day within the period of 28 days. Group III was administered a combination of omeprazole and Symbiter® multiprobiotic. Group IV was administered a combination of omeprazole and Apibact® multiprobiotic.

The activity of superoxide dismutase in cells was determined by Chevars et al. . The catalase activity in cells was determined by Korolyuk et al. . Statistical processing of the results was performed using the "Statistica 7.0" software.

Results: The activity of SOD and catalase in the gastric mucosa of rats after 28 days of omeprazole administration increased compared to the control. Probiotics reduced the activity of SOD compared to the group of rats where omeprazole only was administered. The catalase activity in the gastric mucosa of rats which were jointly administered omeprazole and multiprobiotics for 28 days did not statistically significantly differ from the similar index in the control group.

Conclusions: Prolonged gastric juice hypochlorhydria led to depletion of antioxidant protection enzymes. Multiprobiotics reduced the manifestation of the inflammatory process in the gastric mucosa.

KEY WORDS: hypochlorhydria, proton pump blockers, inflammatory process

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INTRODUCTION

A special feature of many digestive tract organs' diseases is their recurrent nature, as well as their treatment failure in a number of diseases and impossibility to achieve complete clinical remission. Thus, in chronic stomach erosions only half of cases manage to achieve a complete recovery [1]. As for atrophic gastritis with hypo- or anacidity of gastric juice, the picture is even worse. After all, the loss of parietal cells is irreversible, so the treatment of such gastritis is symptomatic and aimed at stopping the disease progression. The use of drugs with antioxidant properties will prevent the pathological process development, as it is noted in the literature [2].

We studied the digestive tract motility functioning under the conditions of prolonged hypacidity of gastric juice and showed the development of inflammatory process in the mucous membrane of the stomach, which led to the suppression of spontaneous and stimulated contractile activity in it. Significant restoration of the stomach motility against the background of omeprazole and multiprobiotics simultaneous administration led us to study the mechanism of the revealed phenomenon [3].

THE AIM

The aim of our work was to study the activity of superoxide dismutase and catalase in the mucous membrane of the stomach in rats under long-term administration of omeprazole and combined administration of omeprazole and multiprobiotics Symbiter and Apibakt.

MATERIALS AND METHODS

The study was carried out on 40 white non-linear male rats with an initial weight of 160-180 g which were kept in the accredited vivarium of the Educational and Scientific Center "Institute of Biology" at Taras Shevchenko National University of Kyiv in compliance with the "Standard rules for the ordering, equipping and maintenance of experimental biological clinics (vivariums) ". All experiments were carried out in compliance with the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment".

All animals were divided into 4 experimental groups. Group 1 of animals served as a control. They were administered intraperitoneally (i/p) 0.2 ml and orally (p/o)

0.5 ml of water for injection within 28 days. Animals of group 2 received omeprazole and 0.5 ml of water for injection once a day for 28 days. Animals of group 3 were co-administered omeprazole and Symbiter® acidophilic concentrated (Simbiter) multi-probiotic once a day for 28 days. Animals of group 4 were administered omeprazole and Apibact® (Apibact) multibiotic simultaneously once a day within 28 days.

Omeprazole (manufactured by "Sigma-Aldrich", USA) was injected intravenously in a dose of 14 mg / kg, dissolved in 0.2 ml of water for injection. Multiprobiotics Simbiter and Apibact (manufactured by OD Prolisok, Ukraine) were administered in combination with omeprazole p/o at a dose of 140 mg / kg (1.4 * 1010 CFU / kg). Multiprobiotics were dissolved in 0.5 ml of water for injection.

The activity of superoxide dismutase (KF 1.15.1.1) in cells was determined by Chevars et al. [4]. The method is based on the ability of superoxide dismutase to compete with nitro blue tetrazolium (NBT) for superoxide anions formed as a result of aerobic interaction of the reduced form of nicotinamidadienedin dinucleotide and phenazine methasulphate (PMS). As a result of this reaction, NBT is restored with the formation of hydrazine tetrazolium. In the presence of superoxide dismutase, the percentage of NBT recovery is reduced.

In a sample containing 0.15 M phosphate buffer, an aliquot of blood serum, cell lysate or mucous membranes homogenate (0.5 mg of protein) was added, the total volume of the test was 0.5 ml. To the sample 1 ml of reagent I was added (57 μM NST, 16 μM FMS to 0.15 M phosphate buffer with EDTA, pH = 7.8). Immediately, absorbance of samples was measured at λ = 540 nm with a spectrophotometer (SF-46, LOMO, Russia). Then, 0.035 ml of reagent II (98.5 μM NAD.H with Tris-EDTA buffer, pH = 8.0) was added to each sample, put in the dark place, and the extinction was re-determined in 10 minutes under the same conditions. Samples were kept at 30° C. The formula was used to calculate the percentage of NBT recovery degree inhibition in the sample:

E= ΔEzero x 50, where:

ΔEstud x 100 x t x a

E – superoxide dismutase activity;

ΔEzero – sample extinction before reagent II adding;

ΔEstud – sample extinction after reagent II adding;

50/100 – 50% blocking of NBT recovery reaction;

a –protein content in the sample, mg;

t –incubation period 10 min.

The enzyme activity was expressed in standard units per minute per 1 mg of protein.

The catalase (KF 1.11.1.6) activity in cells was determined by Korolyuk et al. [5]. The principle of the method lies in the fact that catalase destroys the substrate H_2O_2, the undamaged part of hydrogen peroxide, when in contact with molybdenum salts, forms a stable colored complex.

In a test tube, 2 ml of a 0.03% solution of hydrogen peroxide was added. The reaction was started by adding 0.1 ml of blood serum, cell lysate or mucosal homogenate (0.1 mg of protein). In the control sample, instead of protein, 0.1 ml of distilled water was added. Samples were kept at room temperature for 10 min, the reaction was stopped by adding of 1 ml of 4% ammonium molybdate. The coloration intensity was measured with a spectrophotometer (SF-46, LOMO, Russia) at λ = 410 nm against a control sample, in which, instead of hydrogen peroxide, 2 ml of H_2O was added.

The catalase activity was calculated according to the formula:

E= (Acontr – Astud) , where:

K x t x a

E – catalase activity;

Acontr і Astud – control and studied samples' extinction;

K – millimolar extinction factor of hydrogen peroxide, which equals 22.2 x 103 мМ-1 x cm-1;

t – incubation period 10 min;

a – protein content in the sample, mg.

The activity was measured in nM H_2O_2 in 1 min per 1 mg of protein.

Statistical processing of the results was performed using the Statistica 7.0 software. Since the obtained data were normally distributed, we determined the mean (M) and the standard error of the mean (m) and compared the samples by means of the Student's t-criterion.

RESULTS

Significant changes in the activity of antioxidant enzymes in the rat stomach mucosa after prolonged administration of omeprazole were observed.

The activity of SOD and catalase in the rat mucous membrane after 28 days of omeprazole administration increased by 446.7% ($p < 0.001$) and 26.3% ($p < 0.05$), respectively, compared to the control (fig. 1A, 1B).

In the mucous membrane of the stomach in rats, which were administered omeprazole and Symbiter® multiprobiotic and omeprazole and Apibact® multiprobiotic in combination, the activity of SOD reduced by 57.% ($p < 0.05$) and 34.1% ($p < 0.01$) over the course of 28 days, compared to the group of rats, which were administered omeprazole only (fig. 1A). However, it remained above this index in the control. The activity of SOD under the conditions of of omeprazole and Symbiter® multiprobiotik combined administration was 146.7% ($p < 0.05$), while under the conditions of omeprazole and Apibact® multiprobiotik combined administration 260% ($p < 0.05$) it was higher compared to the control.

The catalase activity in the mucous membrane of the rat stomach, which was administered with omeprazole and multiprobiotics in combination within 28 days, was not statistically reliably different from that of the studied index in the control (fig. 1B).

DISCUSSION

The data we obtained earlier on the significant increase in the content of TBA-reactive substances and NO products is the evidence of oxidative / nitrosative stress development

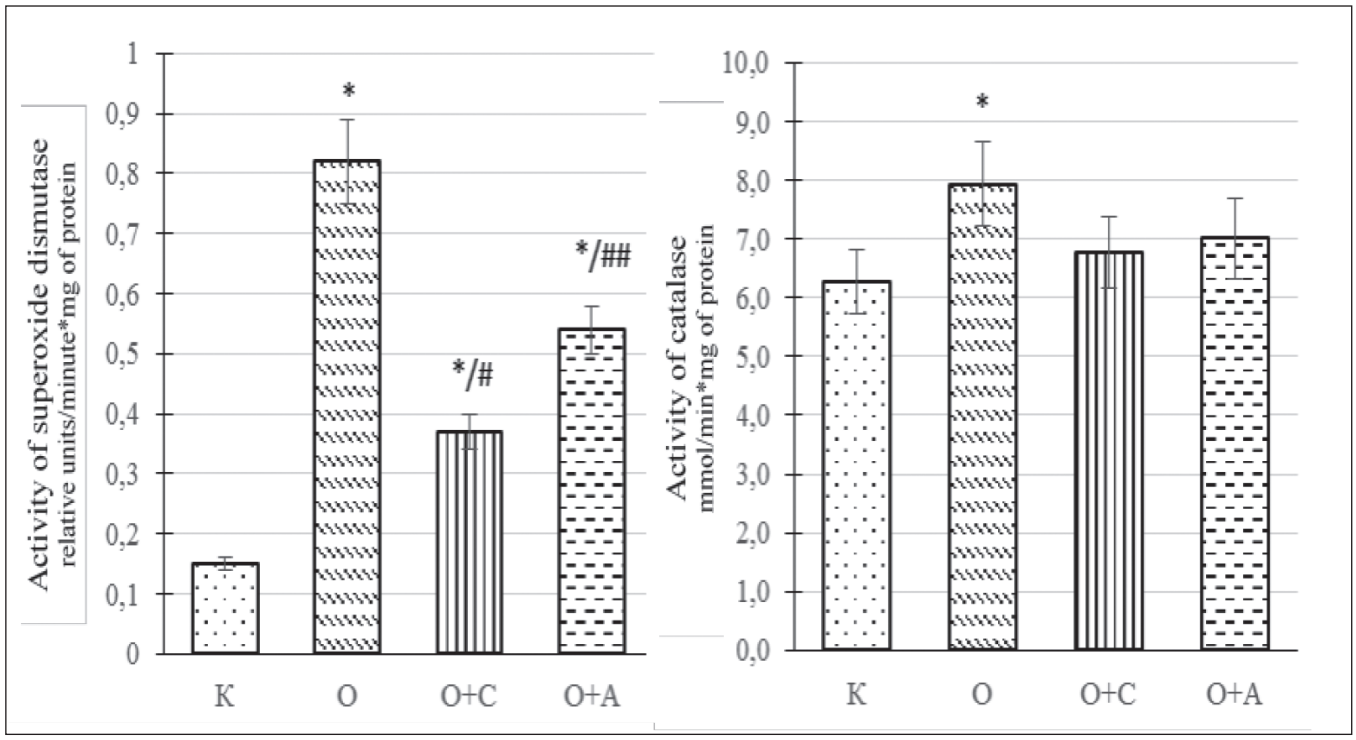

Fig. 1. Activity of superoxide dismutase (A) and catalase (B) in the mucous membrane of the rat stomach, (M + m):
K – control group (n=10); O –group of rats after 28-days of omeprazole administration (n=10); O+C – group of rats after 28-days of the combined omeprazole and Symbiter® multiprobiotic administration (n=10); O+A – group of rats after 28-days of the combined omeprazole and Apibact® multiprobiotic administration (n=10);
* – $p<0.05$ compared to the control;
# – $p<0.05$, ## – $p<0.05$ compared to the group of rats after omeprazole administration.

in the mucous membrane of the stomach under prolonged gastric hypochlorohydria [1].

After 28 days of HCl secretion inhibition, we observed an increase in the activity of superoxide dismutase in the gastric mucous membrane, indicating a significant intensity of the inflammatory process in the stomach, oxidative / nitrosative stress is the result of an imbalance in the antioxidant support system.

Significant increase in the SOD activity was the result of an increase in the formation of oxygen active forms due to stimulation of cytokines by TNF-α, IL-1β and INF-γ [6], which, according to our data, grew after 28 days of omeprazole administration [6, 7].

The inflammatory process is the result of dysbiotic changes in the stomach, which is normally weakly inhabited by microorganisms, and the proximity of target cells (ECL cells and parietal cells) to gastrin and its long hypersecretion.

Under these conditions, an excessive amount of hydrogen peroxide may accumulate in the mucous membrane of the stomach, as the catalase activity that restores it was growing insignificantly.

Our data on the antioxidant properties of multiprobiotics are confirmed by other authors who report the ability of different lactic acid bacteria strains to suppress the lipid peroxidation (LPO) processes and to capture and neutralize free radicals [7, 8]. In addition, it has been shown that Apibact® multiprobiotic reduces the LPO products content in the pancreas and liver of rats under the conditions of prolonged gastric juice hypoacidity caused by omeprazole [9].

CONCLUSIONS

1. Prolonged hypochlorhydria of gastric juice resulted in changes in the functioning and exhaustion of antioxidant support enzymes: compared to the control, the activity of SOD and catalase in the stomach mucous membrane increased by 446.7% ($p<0.001$) and by 26.3% ($p<0.05$).
2. Prolonged administration of multiprobiotic drugs against the background of gastric hypochlorohydria significantly reduced the manifestation of inflammatory process in the stomach mucous membrane, which manifested itself in normalization of antioxidant system enzymes activity.

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Prospects for further research are the study of superoxide dismutase and catalase activity in the mucous membrane of the large intestine in rats under the long-term administration of omeprazole and combined administration of omeprazole and multiprobiotics.

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REVIEW ARTICLE

THE ROLE OF INVASIVE AND NON-INVASIVE MEASUREMENTS OF HVPG IN DECISION MAKING IN PATIENTS WITH PORTAL HYPERTENSION AND ESOPHAGEAL VARICES

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ABSTRACT

Many researchers and clinicians have taken the value of hepatic venous pressure gradient (HVPG) as an essential prognostic factor in subjects with chronic liver disorders. And HVPG alterations characterize a predictive value in subjects at the beginning of the disease (HVPG 6 – 10 mmHg) as well as in subjects in whom hemodynamically significant portal hypertension has developed (HVPG ≥ 10 mmHg).

Our review aims to present the feasibility and applicability of HVPG in modern clinical practice in patients with liver cirrhosis, including invasive and non-invasive methods. HVPG measurement is a feasible method with a favorable safety profile. However, hemodynamically significant portal hypertension also might be determined using non-invasive options as elastography, magnetic resonance imaging, and indices derived from laboratory parameters, e.g., aspartate aminotransferase-to-platelet ratio, platelet count/spleen diameter ratio, or VITRO score. Hepatic vein catheterization with the evaluation of HVPG is the current gold standard for determining portal pressure; however, new non-invasive techniques are nowadays more frequently used.

KEY WORDS: elastography, hepatic venous pressure gradient, wedged hepatic vein pressure, spleen stiffness

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INTRODUCTION

Many researchers and clinicians have taken the value of hepatic venous pressure gradient (HVPG) as an essential prognostic factor in subjects with chronic liver disorders [1]. HVPG alterations characterize a predictive value in subjects at the beginning of the disease (HVPG 6 – 10 mmHg) as well as in subjects in whom hemodynamically significant portal hypertension has developed (HVPG ≥ 10 mmHg). In various scenarios, HVPG values are strictly linked to clinical outcomes. The course of esophageal varices, the risk of ascites and encephalopathy as well as the risk of hepatocellular carcinoma development are associated with HVPG in individuals with liver cirrhosis [2-4].

Additionally, subjects responding to pharmacotherapy of portal hypertension (e.g., decrease in HVPG values > 20% or ≤ 12 mmHg) characterized a clear risk drop of portal hypertensive complications. They also exhibited a better prognosis [5]. Moreover, early HVPG assessment is a useful marker when used in the course of acute variceal bleeding [6]. Other indications for HVPG measurement are displayed in Table 1.

THE AIM

Our review aims to present the feasibility and applicability of HVPG in modern clinical practice in patients with liver cirrhosis, including invasive and non-invasive methods.

REVIEW AND DISCUSSION

HVPG IN COMPUTATIONAL MODELING

Despite the abovementioned utility of HVPG, we still do not fully acknowledge the influence of various factors involved in the cirrhosis pathogenesis and progression on HVPG values. Therefore, Tang et al. developed a computational model of hepatic circulation [7]. They reaffirmed that the HVPG value is useful in evaluating portal hypertension evoked at the sinusoidal and postsinusoidal level rather than at the presinusoidal level. Nonetheless, several factors impact the HVPG measurement accuracy, e.g., the resistance of presinusoidal portal vessels or the flow in portosystemic collaterals.

HVPG INVASIVE MEASUREMENT

Hepatic vein catheterization with HVPG assessment is the current gold standard in verifying portal pressure. HVPG is determined as the difference between the wedged hepatic venous pressure (WHVP) and the free hepatic venous pressure (FHVP). Worth stressing is the fact that WHVP is assessed by occluding the hepatic vein. Blood flow blockage causes pressure equalization with the preceding vascular territory (hepatic sinusoids). This means that WHVP is a surrogate of hepatic sinusoidal pressure, not portal pressure. In healthy subjects, WHVP is only a bit lower (by ~1 mmHg) than portal pressure; however, in cirrhosis, WHVP gives an exact estimate of portal pressure. It was confirmed both for alcoholic and viral cirrhosis. FHVP corresponds to the not occluded hepatic vein pressure [8].

PROCEDURE DETAILS

We obtain FHVP by maintaining the catheter's tip free in the hepatic vein at 2 – 4 cm from its orifice. FHVP value ought to resemble the value of inferior vena cava pressure. A difference of more than two mmHg suggests that the catheter is positioned inappropriately or that a hepatic vein obstruction exists.

WHVP is assessed in an occluded hepatic vein. This is obtained by wedging the catheter tip in a small-diameter branch of the hepatic vein or by inflating a balloon at the catheter's tip. Adequate occlusion of the hepatic vein is confirmed by slowly injecting 5 mL of contrast medium into the vein without observing reflux of the contrast or its washout through communications with other hepatic veins. Occlusion of the hepatic vein by balloon inflation is the preferred technique. The volume of liver circulation sensed by this method is much larger than that attained by wedging the catheter, which reduces measurement variability. Using end-hole, non-balloon catheters is associated with high HVPG variability between various hepatic veins. WHVP should be measured until the value remains stable, preferably for at least 40 seconds. All measurements should be performed at least twice, and all measurements should be recorded. Table 2 presents the elements which should be included in the result. The example procedure is illustrated in Figure 1.

PROCEDURE SAFETY

HVPG measurement is a procedure with a favorable safety profile. Serious complications are mostly restricted to local injuries at the puncture site (femoral, jugular, or antecubital veins) and include hematoma and bleeding, or rarely — arteriovenous fistula or Horner syndrome (in the case of jugular puncture). Ultrasonographic guidance might be additionally used when available, as this tool considerably reduces the risk of procedural complications. The catheter advancement via the right atrium might induce supraventricular arrhythmias (mainly ectopic beats), but in over 90% of cases, they are self-limited.

Although coagulation disorders are common in patients with cirrhosis, only cases of severe thrombocytopenia (platelet levels $< 20 \times 10^9$/L) or a low prothrombin ratio (below 30%) call for the replacement of platelets or transfusion of fresh frozen plasma. The procedure carries only little discomfort. Carried out under moderate conscious sedation (0.2 mg/kg intravenous midazolam, which does not influence HVPG measurement), the procedure's acceptability is comparable to that of upper gastrointestinal tract endoscopy [8].

PROCEDURE LIMITATIONS

Rossle et al. systematically evaluated FVHP measurement accuracy [9]. The study showed that, due to the hepatic vein's conical shape, pressure recordings in the free hepatic vein are substantially affected by the catheter's location. Repeated measurements may be biased by different locations of the catheter's tip and are succumb to manipulation. The authors showed that discrepancies between two locations might exceed the pharmacologic intervention's expected effects (10% – 25% reduction in the HVPG value), bringing into question the reliability of the procedure using FHVP as an internal reference for HVPG. Therefore, it was recommended to simultaneously measure pressures in the IVC at the level of the hepatic veins' entrance and to use this recording when the difference between the two pressures is above two mmHg [8].

Also, Silva-Junior et al. focused on the accuracy of the technique. Authors proved that WHVP/FHVP values were more adequate in prognosis determination than WHVP/inferior vena cava pressure values [10].

Maruyama et al. analyzed the occurrence and hemodynamic features of high-risk esophageal varices with low HVPG values [11]. In the studied population, authors identified 16.4% of subjects with high-risk esophageal varices and HVPG values below 10 mmHg. The venous-venous communication (VVC) incidence was higher in subjects with HVPG values below 10 mmHg ($p < 0.01$). Subjects with a red sign characterized lower HVPG values (13.3 ± 4.5 mmHg) but advanced left gastric vein hemodynamics (velocity 13.2 ± 3.8 cm/s; flow volume 217.5 ± 126.6 mL/min). On the other hand, subjects without a red sign characterized higher HVPG values (16.2 ± 4.6 mmHg, $p < 0.01$) and worse left gastric vein hemodynamics (10.9 ± 2.3 cm/s, $p < 0.01$; 160.1 ± 83.1 mL/min, $p = 0.02$).

Kim et al. characterized the significance of the classified hemodynamic stage on the basis of HVPG values in subjects with portal hypertension [12]. The following two hemodynamic stage classifications were applied: classification 1 (6 – 9, 10 – 12, 13 – 16, 17 – 20, and > 20 mmHg) and classification 2 (6 – 12, 13 – 20, and > 20 mmHg). Death rates in classification 1 subgroups were 6.3%, 6.9%, 18.0%, 15.6%, and 34.4%, respectively ($p < 0.01$). Also, in classification 2, subgroups mortality rates differed significantly ($p < 0.05$). Interestingly, in the multivariable model, only classification 2 was a significant prognostic factor in mortality.

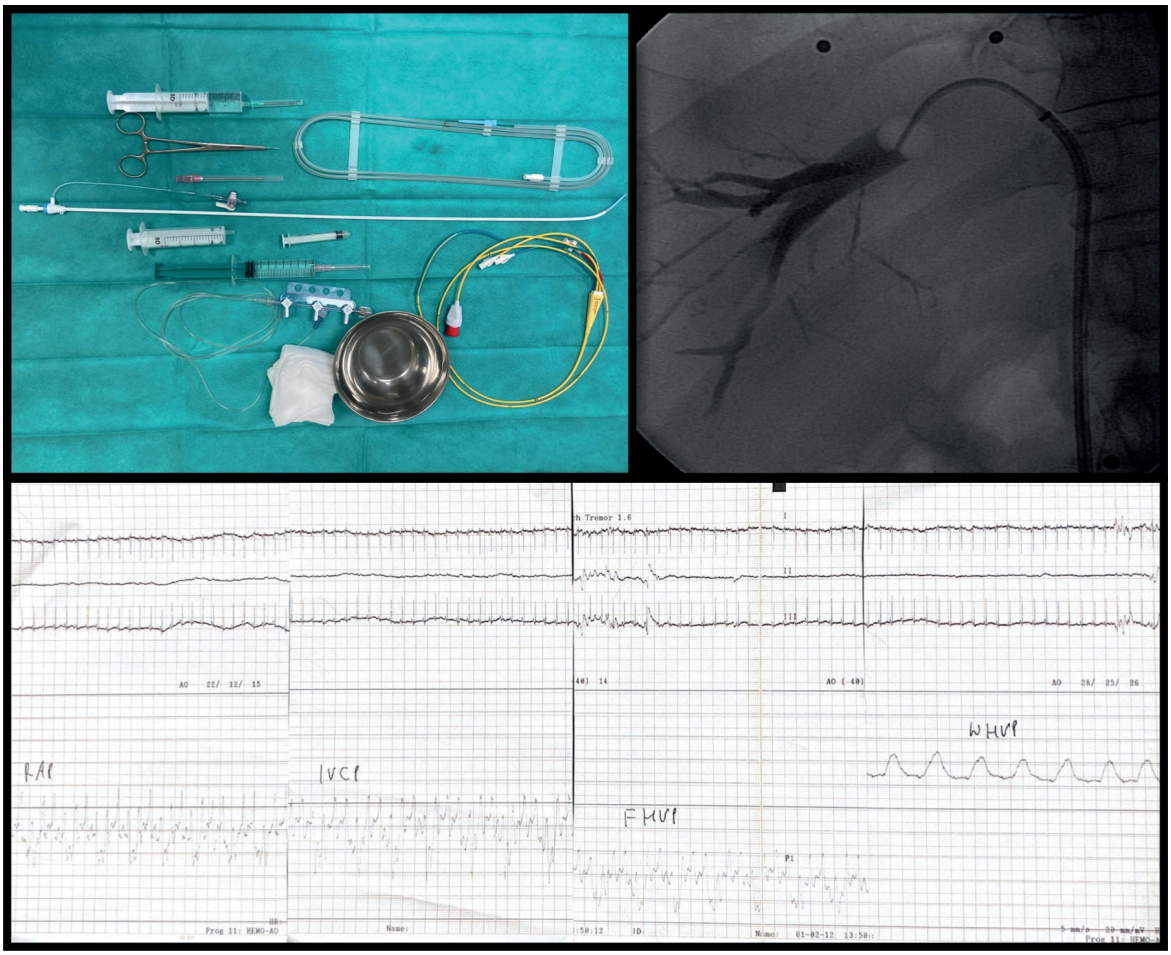

Fig. 1. The example hepatic venous pressure gradient (HVPG) measurement procedure.

ENDOSCOPIC ULTRASOUND-GUIDED PORTAL PRESSURE GRADIENT MEASUREMENT

Huang et al. characterized the endoscopic ultrasound-guided portal pressure gradient measurement. The procedure was done with the use of a linear echoendoscope, a 25G needle, and a novel compact manometer. Both portal vein and hepatic vein (or inferior vena cava) were reached through a transgastric (or transduodenal) approach. Twenty-eight subjects underwent endoscopic ultrasound-guided portal pressure gradient measurement. Technical success was 100%, and authors registered no complications. HVPG values ranged from 1.5 –19 mmHg and highly correlated with clinical features of portal hypertension such as thrombocytopenia ($p = 0.04$), varices presence ($p < 0.01$) or portal hypertensive gastropathy ($p < 0.01$) [13].

NON-INVASIVE MEASUREMENT TECHNIQUES

LABORATORY PARAMETERS

Several non-invasive methods were evaluated as potential screening options for esophageal varices. Some simple parameters were identified as being associated with the presence of varices such as platelet count, prothrombin activity, albumin, alanine aminotransferase, spleen diameter, portal vein diameter, ascites, telangiectasias, or the Child-Pugh classification. However, their utility in predicting esophageal varices was not satisfactory.

Aspartate aminotransferase-to-platelet ratio index (APRI) is another option. It was first established in predicting liver fibrosis in subjects with hepatitis C [14]. Mattos et al. disclosed that APRI characterized 64.7% sensitivity and 43.2% negative predictive value in predicting esophageal varices in subjects with cirrhosis [15]. More recently, Mandal et al. demonstrated that APRI with a threshold of 0.908 characterized 87.3% sensitivity, 71.4% specificity, 92% positive predictive value, and 60% negative predictive value [16]. Consequently, APRI might be a helpful tool in indirectly verifying the esophageal varices risk in subjects with liver cirrhosis.

Another proposed index, platelet count/spleen diameter ratio (PC/SD) with the threshold of 909, characterized 100% negative predictive value. The justification for introducing the PC/SD ratio was the idea to correct thrombocytopenia, which is frequently observed in liver diseases. PC/SD ratio was also confirmed to be a helpful marker to follow-up patients without varices. Still, validation results were not as astounding as in the original research (91.5% sensitivity, 87% negative predictive value) [17]. This was also verified in more recent studies [18].

Table 1. Clinical applications of hepatic venous pressure gradient (HVPG) measurement

Diagnosis of portal hypertension
Classification of portal hypertension (prehepatic, intrahepatic, posthepatic)
Assessment of disease severity and prognosis
Assessment of new therapeutic agents
Response to therapy for portal hypertension

Table 2. The measurement of hepatic venous pressure gradient.

Key data included in the study protocol
1. Access route
2. Type of catheter(s)
3. Hepatic veins used for pressure measurements (right vs middle vs left)
4. FHVP
5. WHVP
6. HVPG
7. Inferior vena cava pressure
8. Right atrial pressure
9. Complications
10. Additional comments

FHVP – free hepatic venous pressure; WHVP – wedged hepatic venous pressure; HVPG – hepatic venous pressure gradient

Hametner et al. evaluated von Willebrand factor antigen (vWF-Ag) to thrombocyte ratio (VITRO score) as a potential marker in predicting hemodynamically significant portal hypertension [19]. Area under the curve (AUC) values showed 0.86 for VITRO score, 0.79 for vWF-Ag, and 0.62 for APRI.

ULTRASOUND

A doppler ultrasound exam is definitely helpful in the assessment of blood flow in the portal and splanchnic vessels as well as in the imaging of morphological abnormalities co-existing with portal hypertension (e.g., splenomegaly, dilatation of the portal vein system, or development of portosystemic collaterals). The damping index (showing changes in the doppler hepatic vein waveform) corresponds with hemodynamically significant portal hypertension and HVPG values (together with HVPG changes after treatment) [20]. An approach to measuring the resistance of the splenic artery using the splenic doppler pulsatility index together with the portal blood flow is also a reliable option. The obtained results correlated more strongly with HVPG values than any other doppler measurements. Lee et al. proved that the splenic arterial resistive index characterized an improved diagnostic performance in comparison with the liver stiffness evaluated in shear wave elastography [21].

Contrastenhanced ultrasound measurements have recently presented interesting data. The application of subharmonic aided pressure estimation (SHAPE) with the use of perflubutane microbubbles characterized a pretty good correlation with HVPG values. This method was validated in a large multicenter study in the USA (NCT02489045) [21]. Subjects at increased risk for variceal bleeding (HVPG ≥ 12 mmHg) had a higher mean SHAPE gradient compared to subjects with lower HVPG values (0.79 dB ± 2.53 vs. -4.95 dB ± 3.44; $p < 0.01$). The sensitivity was 90%, and the specificity was 80%. The SHAPE gradient between the portal vein and the hepatic vein correlated well with the HVPG values ($r = 0.68$).

ELASTOGRAPHIC METHODS

Transient elastography was the first method introduced to assess liver stiffness, focused on estimating liver fibrosis. First, transient elastography was applied as an option to invasive liver biopsy for fibrosis staging in subjects with liver disease, especially hepatitis C. Afterwards, transient elastography was proven to characterize a high accuracy in liver cirrhosis recognition. Nowadays, it is generally accepted that transient elastography measurements can also be correlated with the degree of portal pressure. Many researchers tried to overcome liver stiffness limitations on detecting patients with HVPG > 12 mmHg, or those with large esophageal varices, by estimating the spleen stiffness. Spleen enlargement is one of the essential diagnostic signs of advanced liver disease. It would be not irrational to hypothesize that, in contrast to liver stiffness, which seems to correlate well with portal pressure only at the initial stages of portal hypertension (HVPG < 10 mmHg), where the fibrotic component dominates, the spleen stiffness may correlate better with portal hypertension at more advanced stages, when the hyperdynamic circulation and increased portal venous inflow, participates. Studies showed that the combination of liver stiffness and spleen stiffness increased the accuracy of diagnosing patients with advanced compensated cirrhosis not needing screening endoscopy. Karagiannakis et al. disclosed that sequential application of the liver and spleen shear wave elastography predicted hemodynamically significant portal hypertension (HVPG value > 10 mmHg) with high accuracy [22].

In another study, the following parameters were analyzed: liver stiffness, spleen stiffness, PC/SD, liver stiffness-spleen diameter to platelet ratio score, and variceal risk index [18]. The authors observed significant differences among subjects with or without gastroesophageal varices. The optimal threshold for diagnosing the gastroesophageal varices

presence was 12.3 kPa in case of liver stiffness and 27 kPa in case of spleen stiffness. However, diagnostic accuracy was moderate (AUC: 0.671 and 0.624, respectively).

In a meta-analysis, Song et al. assessed the association between spleen stiffness determined in transient elastography and HVPG values. They also evaluated the accuracy of spleen stiffness measurement in recognizing hemodynamically significant portal hypertension [23]. The agreement between spleen stiffness and HVPG values was good, and spleen stiffness characterized good sensitivity and specificity. Unfortunately, the various threshold values among analyzed studies might hamper the significance of obtained results in clinical practice.

In another meta-analysis, Manatsathit et al. analyzed liver stiffness, spleen stiffness, and liver stiffness-spleen size-to-platelet ratio risk score in detecting esophageal varices and high risk/clinically significant esophageal varices [24]. Spleen stiffness and liver stiffness-spleen size-to-platelet ratio risk score were better than only liver stiffness measurement in detecting esophageal varices. These parameters characterized better sensitivity (0.90 and 0.91 vs. 0.85), specificity (0.73 and 0.76 vs. 0.64), odds ratio (3.24 and 3.35 vs. 2.26), and AUC (0.89 and 0.85 vs. 0.82). For high risk/clinically significant esophageal varices, spleen stiffness had the highest sensitivity (0.87), followed by liver stiffness (0.85) and liver stiffness-spleen size-to-platelet ratio risk score (0.82); however, spleen stiffness had the lowest specificity (0.52), odds ratio (2.09), and AUC (0.81), whereas liver stiffness-spleen size-to-platelet ratio risk score had the highest specificity (0.77), odds ratio (2.74), and AUC (0.86).

And lastly, Piecha et al. disclose that subjects with reducing liver stiffness on the treatment with propranolol characterized a decreased risk for transplantation or death compared to subjects with elevated liver stiffness irrespective of HVPG values [25].

MAGNETIC RESONANCE IMAGING

Hemodynamic parameters obtained in magnetic resonance imaging correlate with HVPG values. Gouya et al. proved that the azygos flow determined in MRI correlated well with HVPG values (AUC 0.96, 95% CI 0.91 – 1.00) in subjects with liver cirrhosis due to hepatitis C or alcohol [26]. In a multicenter study, Palaniyappan et al. showed that a predictive model, including splenic artery velocity, significantly correlated with HVPG values and characterized a good HVPG value predictability in a validation group [27].

COMPUTED TOMOGRAPHY

Deng et al. performed a metaanalysis, including 17. They showed that computed tomography sensitivity and specificity in detecting esophageal varices of any size were 87% and 80%, respectively. For highrisk esophageal varices, the values were 87% and 88%, respectively [28]. Moreover, in subjects with liver cirrhosis, splenic clearance determined in computed tomography perfusion imaging characterized excellent performance in recognizing the value of HVPG ≥ 12 mmHg (94% sensitivity, 100% specificity) [29].

CONCLUSIONS

Over 20 years ago, the measurement of HVPG was applied in managing subjects with liver cirrhosis, mainly in the prevention of the first variceal bleeding. HVPG measurement is a feasible method with a favorable safety profile. However, HVPG measurement is not widely available in clinical practice due to its invasive character and limited feasibility. Additionally, hemodynamically significant portal hypertension may be evaluated using non-invasive methods, such as transient elastography.

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Conflict of interest

The Authors declare no conflict of interest.

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REVIEW ARTICLE

CHALLENGES OF CLASSIFICATION OF STAND-ALONE SOFTWARE AS A MEDICAL DEVICE

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ABSTRACT

Through a broad literature review, analysis of EU, USA, Ukraine regulation acts, scientific research, and opinions of progressive-minded people in this sphere, this paper provides a guide to understanding the essence of classification of stand-alone software with medical purpose and specifics of its regulation. This research is based on dialectical, comparative, analytic, synthetic, and comprehensive methods.

KEY WORDS: apps, applications, software, medical devices, classification, medical devices directive, medical devices regulation, legislation, classification, authorized body

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INTRODUCTION

Thousands of applications, algorithms, and pieces of software have been developed in the healthcare field over the past few years, all with the aim of improving patient health and assisting doctors in clinical decisions. Apps and computer programs are designed to do everything from track menstrual periods to help anesthesiologists during surgery. In many cases, they have also been proposed to help guide decision-making when tumors should be biopsied or when medication should be delivered [1].

As the number of products has increased, the authorities in different countries work hard to develop and implement relevant legislation and guidelines. Furthermore, one of the most challenging matters in this regard is stand-alone software classification, and it will be the focus of this particular research.

MATERIALS AND METHODS

Through a broad literature review, analysis of EU, USA, Ukraine regulation acts, scientific research, and opinions of progressive-minded people in this sphere, this paper provides a guide to understanding the essence of classification of stand-alone software with medical purpose and specifics of its regulation. This research is based on dialectical, comparative, analytic, synthetic, and comprehensive methods.

REVIEW AND DISCUSSION

I. Stand-alone software classification rules: EU, USA, and Ukrainian approaches

The first question that has to be answered by the manufacturer of stand-alone software in the health sphere – is this software a medical device or not? This question got significant scientific attention and was partly covered in our previous researches [2-9]. That is why we will move further, leaving these basic points.

Once a stand-alone software is qualified as a medical device, the next question to be answered is into what classification group it should fall.

In the European Union (hereinafter – EU), the documents that regulate this question are Medical Device Directives (MDD): AIMDD 90/385/EEC; MDD 93/42/EEC; IVDMDD 98/79/EC (hereinafter – MDD). The MDD defines possible classes for medical devices as being either Class I, IIa, IIb, or III and provides a set of rules for deciding on the appropriate classification for a device. These rules, called "implementing rules", are contained in Annex IX of the MDD [10].

Section 1.4 of Annex IX states clearly that 'stand-alone software is considered to be an active medical device':

"Any medical device operation of which depends on a source of electrical energy or any source of power other than that directly generated by the human body or gravity and which acts by converting this energy. Medical devices intended to transmit energy, substances, or other elements between an active medical device and the patient, without any significant change, are not considered to be active medical devices. The stand-alone software is considered to be an active medical device". [10].

This means that implementing rules 9, 10, 11, and 12 may apply, depending on the stand-alone software's function and purpose.

The new Medical Device Regulation, published in April 2017 and replacing the MDD in May 2020, puts more emphasis on software [11].

General-purpose software or software for life-style and well-being purposes is explicitly excluded from the MDR. Compared to the MDD, there is an additional classification rule (rule 11) for software in the MDR that covers other types of software.

Thus, Rule 11 states that software intended to provide information which is used to make decisions with diagnosis or therapeutic purposes is classified as class IIa, except if such decisions have an impact that may cause:

death or an irreversible deterioration of a person's state of health, in which case it is in class III; or

a serious deterioration of a person's state of health or surgical intervention, in which case it is classified as class IIb.

Software intended to monitor physiological processes is classified as class IIa, except if it is intended for monitoring of vital physiological parameters, where the nature of variations of those parameters is such that it could result in immediate danger to the patient, in which case it is classified as class IIb.

All other software is classified as class I [11].

It is important to say that under the MDD, the majority of software falls under Class I, the more stringent MDR requirements may determine the reclassification of some mHealth Apps into a higher risk class. Examples of up-classification from Class I to Class IIa are an app that measures reaction time to help diagnose a concussion and an app that calculates the risk of having a heart attack in the next ten years based on cholesterol levels. An example of up-classification from Class I to Class IIb is an app that provides information on the required dose of medicine (an anticoagulant) based on the measurement of the international normalized ratio (INR) value for blood clotting. In this case, it was assumed that the app calculates this value using an algorithm. One app was classified as Class I under the MDD but will be a Class III medical device under the MDR. This is an app predicting the three-month mortality risk in patients with chronic liver disease. This score is used for prioritization of donor organ allocation to patients awaiting liver transplantation [12, p.19].

Moving from Class I to a higher class implies notified body involvement in the conformity assessment, which represents a heavier burden for mHealth Apps developers in terms of budget and time planning [13].

In the USA, as in the EU, medical devices software could fall under all types of classes. Thus, The Policy for Device Software Functions and Mobile Medical Applications, issued by the FDA, defines that for device software functions, manufacturers must meet the applicable device classification requirements. Thus, If the device, on its own, falls within a medical device classification, its manufacturer is subject to the requirements associated with that classification. A device software function, like other devices, may be classified as class I (general controls), class II (special controls in addition to general controls), or class III (premarket approval) [14, p. 10].

For comparison, Ukrainian legislation defines four classes for medical devices: I, IIa, IIb, and III (paragraph 14 of the Technical Regulations, medical devices) [15]. Classification is carried out per the criteria set out in Annex 2. Paragraph 5 of Annex 2 of the Technical Regulation, which stipulates that software that controls a medical device's operation or affects a medical device's use belongs to the same class as this medical device [15]. It follows that software that is part of another medical device has the same class as this medical device. In fact, with this category of software, everything is obvious, and no questions arise. In our opinion, it is much more challenging to classify software that is available to the user as a separate medical device and is not a part of any other "hardware" medical device. In order to determine the class of this type of software, we refer to the general rules of classification of non-invasive (those that do not come into contact with the patient or come into contact only with intact skin) medical devices provided for in paragraphs 9-12 of Annex 2 above. All non-invasive medical devices are in Class I unless the provisions of this section apply [15]. An analysis of the provisions of this section shows that its provisions do not apply to the software. Therefore, stand-alone software belongs to class I (the lowest degree of risk).

It should be noted that in the methodological recommendations on the application of the Technical Regulation on medical devices approved by the Order of the Ministry of Health of Ukraine dated 22.01.2020 № 142, which provide additional clarifications, including the classification of medical devices, no guidelines or ancillary tools that would help with the classification of stand-alone software are not contained [16]. Only in the document Methodical Recommendations "Medical devices. Aids. Producer." [16] explains whether a product, in particular software, can be considered to have a medical purpose (namely, for this purpose, the software belongs to the category of medical devices) or not. Thus, these recommendations clearly state that the product's medical purpose is absent when the software is used to process general patient data [16]. However, this rule in no way affects the fact that individual software belongs to class I.

Summarizing the abovementioned, we can conclude that the Ukrainian approach in stand-alone software classification is not aligned with EU and USA approaches, when stand-alone software could fall under all types of medical device`s classes depending on the level of the risk, where the premarket way of stand-alone software can include involving a notified body and grant their effectiveness and safety for potential customers.

II. Certified in EU and USA stand-alone software: classification and learnings

We have analyzed the directory of certified apps in the EU and USA [17], which includes 213 medical devices from the different standpoints, including classification, summarized results are below (Table 1, Table 2).

As we can see from the table above, the most popular categories of certified stand-alone software are Heart/ Circulatory System, Diabetes, Patient monitoring, and Respiratory system.

In this regard, it is also interesting to compare the number of certified stand-alone software as a medical device

Table 1. Medical area for use

Category	№	%
Heart/Circulatory System	41	19,25
Diabetes	36	16,9
Patient monitoring	29	13,62
Respiratory system	21	9,86
Eyes and Ears	11	5,16
Mental Health and Behavioral Disorders	11	5,16
Genitourinary system	8	3,76
Communication System	8	3,76
Pain management	6	2,82
Sleep management	5	2,35
Musculoskeletal system and connective Tissue	5	2,35
Oncology	5	2,35
Fertility	2	0,94
Epilepsy	2	0,94
Real-time monitoring of tissue oxygen	1	0,47
Infant feeding	1	0,47
Drug delivery	1	0,47
Chronic disease management	1	0,47
Skin and subcutaneous tissue	1	0,47
Medical devices with a few purposes and others	18	8,45

according to the conformity assessment rules and the total number of medical and health software available in the relevant categories in online stores. Thus, as we mentioned above, according to the information provided in our analyzed database, there is 213 certified software that has a medical purpose. In the Google Play store, in section "Applications," category "Medicine," in the list of TOP free applications, there are 200 units, in section bestsellers – 54 units; TOP paid – 188 units. Also, there is a separate category – "Health and Fitness," which offers various fitness programs, pedometers, and fitness trackers, which do not qualify as medical devices [18]. The AppStore also includes several categories of software that can be used for medical purposes: "Health and Fitness," where 234 programs are offered as popular at once; "Medicine" – 240 programs stand out as popular [19]. The Microsoft Store, in turn, also has both categories: "Medicine" – 204 programs and "Health and Fitness" – 739 programs are available [20].

This indicates that the number of stand-alone software that may have a medical purpose, and therefore is required to undergo a proper conformity assessment procedure on the market much more than duly registered and provided in the analyzed database of certified apps. As a result, it is likely that some medical software may pose a risk to human life and health and is a medical device but has not been adequately tested before it is made available to the end-user.

Within the framework of this classification criterion, it is possible to note that the market is not as defined as other goods in the field of software trade because it is sold via the Internet. Moreover, although the stand-alone software in the above pages is distributed by location, i.e., the user is displayed for sale software available at his location. However, this does not preclude the use of VPN technology to hide the location and access the software from "another market".

Besides, a sample analysis of 30 programs from the "Medicine" section of GooglePlay, available to Ukrainian users, showed that none of the programs contains a national mark of conformity that should be applied if such software is classified as a medical device and has passed the conformity assessment [21]. Which in turn, can mean one of two things: either all the software under analysis is not a medical device in the sense of national law, or some of the software is a medical device but has not passed the proper conformity assessment procedure according to the Technical Regulations for Medical Devices.

From this classification, it can be concluded that the vast majority (about 71%) of certified stand-alone software is represented in both the AppStore and Google Play, indicating that there are sufficient staff to develop and maintain multi-platform software (at least for Android and iOS operating systems), which can usually be afforded by medium and large companies that already have a success story on one of the platforms. From these statistics, it can be indirectly concluded that small companies and start-ups cannot afford to go through the conformity assessment procedure (Table 3).

From the analysis, it can be concluded that the vast majority (105 against 20) of medical software, which is

Table 2. Sales market

Market	№	%
Only USA	81	38,74
Only EU	45	21,53
Both EU and USA	83	39,73

Table 3. The way of distribution

The way of distribution	№	%
AppStore, Google Play and website	149	71,59
AppStore and Website	36	17,3
Google Play and Website	13	6,24
Only Website	10	4,87

Table 4. The class of certified stand-alone software EU

Class	№	%
CE I	105	84
CE II a	15	12
CE II b	5	4

Table 5. The class of certified stand-alone software USA

Class	№	%
FDA 510 (k)	135	88,24
FDA 510(k) exempt	4	2,61
FDA class I	6	3,92
FDA class II	8	5,23

designed for the EU market, is classified according to CE I class, which has the lowest degree of risk to humans and provides the most painless procedure for conformity assessment, namely self-assessment and completion declaration, therefore, does not require the involvement of a conformity assessment body (Table 4). The situation is similar to medical software available to users in the US (Table 5). Thus, the vast majority of such software (105) entered the market after submitting form 510 (k). In fact, in this form, the medical software manufacturer must demonstrate that its medical device is as safe and effective as a similar medical device (medical software) that already legally exists on the market [22].

III. Stand-alone software classification: practical issues

Previously we considered what classes of medical devices could be applied for stand-alone software in the EU, USA and Ukraine, analyzed the database of certified stand-alone software in the EU and USA, and now we would like to explore how classification rules work in practice on the example of one stand-alone type software in the category Birth control.

From the database of certified health apps [17], we can see just one software that the FDA cleared in the US and CE-marked in Europe as a medical device – Natural Cycles – Birth Control App [23]. The main functions of this app are 1) Knowledge of when a woman is fertile and when is not; 2) Reliable ovulation detection and predictions; 3) Personal insights around your cycle, enabled by our proprietary algorithm; 4) Useful notifications, including PMS alerts and when your period is due. Natural Cycles was classified as a medical device that falls under Class II a.

Let's see what recommendations regarding classification was provided in the Manual on borderline and classification in the community regulatory framework for medical devices Version 1.22 (05-2019) (Table 6). Even though this document is not legally binding, it was developed by the group that was chaired by the commission and composed of representatives of all member states of the EU, EFTA, and other stakeholders [24]. This Manual provided three examples of the classification of birth control apps.

As we can see, the first, second, and third apps are working in the same way, but the first one is classified as Class I and the second, and the third as Class IIb. The difference is in intended purpose: just facilitate conception or facilitate conception and prevent pregnancy. If the manufacturer declares the purpose of preventing pregnancy – it has to be Class IIb.

Returning to Natural Cycles, this app was classified as Class IIa, and its intended purpose – Birth Control (in this case, it is unclear if it was designed just to facilitate conception or prevent pregnancy as well). Thus, under these four examples, we can see that four apps with the same functions and that can be used for the same purpose could be classified as Class I, Class IIa, or Class IIb depending on the manufacturer's purpose. The only common feature – all of them are qualified as a medical device. Consider-

Table 6. Examples of the classification of birth control apps [24].

Category: Product intended to facilitate conception based on basal body temperature **Description:** The product in question measures the basal body temperature (BBT) orally, records it and uses the daily BBT and menstruation days to track the menstrual cycle and predict ovulation. Once it has enough data from the user, it calculates the user's fertility status based on a validated statistical algorithm as claimed by the manufacturer. The fertility status of the current day is reflected by one of three indicator lights: red (fertile), green (infertile) or yellow (learning phase/cycle fluctuation).The temperature sensor, the activation button (interface), the processor (storing of data and fertility status calculator) and the menstruation and fertility status indicators are all integrated into a single piece of equipment. The product is battery-driven. It does not display the user's temperature and is not intended to allow direct diagnosis or monitoring of vital physiological processes, i.e., it is not intended to be used as an electronic thermometer. It is claimed by the manufacturer to facilitate conception by predicting ovulation. **Outcome:** The product is intended by the manufacturer **to facilitate conception** and **should be qualified as a medical device**. According to clauses 1.2 and 1.4 of chapter I, Annex IX MDD, this device is considered to be an invasive active medical device and should be classified **as class I according to rule 12**	**Class I**
Category: Product intended to facilitate conception and enable contraception based on basal body temperature **Description:** The product in question measures the basal body temperature (BBT) orally, records it and uses the daily BBT and menstruation days to track the menstrual cycle and predict ovulation. Once it has enough data from the user, it calculates the user's fertility status based on a validated statistical algorithm as claimed by the manufacturer. The fertility status of the current day is reflected by one of the three indicator lights: red (fertile), green (infertile) or yellow (learning phase/cycle fluctuation).The temperature sensor, the activation button (interface), the processor (storing of data and fertility status calculator) and the menstruation and fertility status indicators are all integrated into a single piece of equipment. The product is battery-driven. It does not display the user's temperature and is not intended to allow direct diagnosis or monitoring of vital physiological processes, i.e., it is not intended to be used as an electronic thermometer. The product in question is claimed by the manufacturer to facilitate conception and prevent pregnancy. **Outcome:** The product is intended by the manufacturer to (i) facilitate conception or (ii) prevent **pregnancy,** and should therefore be qualified as a medical device. According to clause 4.2 of chapter III, Annex IX MDD, all devices used for contraception or the prevention of the transmission of sexually transmitted diseases are in Class IIb (rule 14). The explanations of rule 14 in the MEDDEV 2.4/1 Rev. 9 emphasize that "the intended uses relate to special cases of human vulnerability that cannot be covered by the normal criteria of time, invasiveness and organic function." Unwanted pregnancy is a case of human vulnerability. According to clauses 1.2 and 1.4 of chapter I, clause 2.5 of chapter II and clause 4.2 of chapter III, Annex IX MDD, and in consideration of the above-mentioned rationale, this device is considered to be an invasive active medical device and should be classified as class IIb according to rule 14.	**Class IIa**
Category: Standalone software application for conception and contraception purposes using data entered by the patient **Description:** The product in question is claimed by its manufacturer to be a natural method of birth control. It is a standalone software application that combines the calendar/rhythm method, the body basal temperature (BBT) method and the cervical mucus method to both (i) prevent pregnancy and (ii) target the most fertile time for getting pregnant. The user enters her data (first day of menstruation, BBT measured with a common thermometer and consistency of the cervical mucus) and obtains the fertility window. **Outcome:** The product is intended by the manufacturer to (i) **prevent pregnancy** or (ii) facilitate conception, and should be qualified as a medical device. According to clause 1.4 of chapter I, clause 2.5 of chapter II and clause 4.2 of chapter III, Annex IX MDD, this standalone software is considered to be an active medical device and should be classified as **class IIb according to rule 14.**	**Class IIb**

ing those mentioned above, the more strange the market situation looks, thus just in Google Play, there are more than 250 woman's apps [25] with similar functions and similar risks for a woman but without any qualification or classification at all.

Thus, we can conclude that qualification of the software as a medical device is challenging, but the next step with classification is difficult and not clear as well. Despite the existing Manual with examples and explanations, there is a lot of stand-alone software with a medical purpose available at the market without necessary control, management, and confirmation of quality and safety for customers. This issue should be considered and resolved to restrict customers from potential harm such software could pose to their life and health.

CONCLUSIONS

Summarizing the above mentioned, it is feasible to make the following conclusions:

1. Under the new EU rules, stand-alone software could be classified as Class I, Class IIa, IIb, or Class III (previously, most of the stand-alone software was classified as Class I). The same approach of classification works in the USA, when stand-alone software may be classified as Class I (general controls), Class II (special controls in addition to general controls), or Class III (premarket approval). Under the Ukrainian legislation, stand-alone software classified as Class I that is not compliant with EU Directive and USA guidelines and practice.
2. New rules of classification for EU manufacturers mean higher costs for development and additional time for

the pre-marker stage, taking into account the involvement of notified bodies in the certification process. It could have a negative impact on small companies and startups with limited resources.

3. The analysis of the certified stand-alone software database allowed to create the next inferences:

- the most popular categories among certified stand-alone software are Heart/Circulatory System, Diabetes, Patient monitoring, and Respiratory system;
- in the analyzed database, there is 213 certified stand-alone software. However, in the online stores, much more stand-alone software in the categories Medicine and Health and Fitness are available, which means that there is no effective control on producers and sales of stand-alone software with medical purposes from the authorities' perspective;
- the vast majority (about 71%) of certified stand-alone software is represented in both the AppStore and the Google Play, indicating that there are sufficient staff to develop and maintain multi-platform software (at least for Android and iOS operating systems), which can usually be afforded by medium and large companies that already have a success story on one of the platforms;
- the vast majority (105 against 20) of medical software, which is designed for the EU market, is classified according to CE I class, which has the lowest degree of risk to humans and provides the most painless procedure for conformity assessment, namely self-assessment and completion declaration, therefore, does not require the involvement of a conformity assessment body.
- most of the medical software available to users in the US (105) was entered the market after submitting form 510 (k)

4. The detailed analysis of applying classification rules to stand-alone software in the category Birth control shows that the four different apps with the same functions and that can be used for the same purpose could be classified as Class I, Class IIa, or Class IIb depending on the manufacturer's purpose. There are a lot of available stand-alone software in the same category and for a similar purpose and with similar functions, available for end users without any certification

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REVIEW ARTICLE

MODERN APPROACHES TO THE FORMATION OF PROFESSIONAL COMPETENCIES OF PHARMACISTS ON ISSUES OF MEDICINES QUALITY ASSURANCE

DOI:10.36740/WLek202102130

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ABSTRACT

The aim: To analyze, summarize and substantiate modern approaches to the formation of the professional competencies of pharmacists on issues of medicine quality assurance in Ukraine.

Materials and methods: In this study, we performed systematic review, systematic and comparative analysis, content analysis, generalization, document analysis, logical and graphical modeling to address those issues.

Conclusions: We showed that the curriculum and program of the «Basic foundations of the functioning of quality systems in pharmacy institutions» thematic improvement cycle for pharmacists have been substantiated and developed. The content of the program provides an opportunity to prepare pharmacists for independent work in the field of implementation and support of effective quality systems in pharmacy institutions and hospital pharmacy services. In the context of substantiation of modern approaches to the preparation of pharmacists in the field of medicines quality assurance, the experience and advantages of such modern forms of training of pharmacists as the use of training bases and blended learning have been studied. The modern approaches to the formation of the professional competencies of pharmacists on issues of medicine quality assurance in Ukraine have been analyzed, summarize and substantiated.

KEY WORDS: pharmacist, quality assurance, pharmacy institution, pharmaceutical education, postgraduate education, continuous professional development

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INTRODUCTION

The effectiveness of the quality systems in pharmacy institutions and hospital pharmacy services depends directly on the availability of competent staff [1, 2]. Therefore, the study of different aspects of medicine's quality assurance should permeate the curricula and programs for pharmacists at all stages of pharmaceutical education and continuing professional development (CPD).

The first and basic stage of preparation of pharmacy professionals in Ukraine is obtaining higher pharmaceutical education, which is carried out in the institutions of higher pharmaceutical (medical) education (IHE). Until 2017, the obtaining of the incomplete higher pharmaceutical education was carried out in the IHE at the accreditation levels I–II according to the educational and professional program (EPP) of pharmacy professional training of the Junior Specialist Degree in speciality 5.120201 «Pharmacy». After mastering this EPP, such professional was awarded the Pharmacist qualification. The obtaining of the complete higher pharmaceutical education was carried out in the IHE at the accreditation levels III–IV according to the EPP of pharmacy professional training of the Specialist Degree in speciality 7.120201 «Pharmacy». After mastering this EPP, such professional was awarded the Provisor qualification and he was granted the right to perform the professional work of the provisor-intern. If desired, the provisor could enter the master program and obtain the Master of Pharmacy Degree in speciality 8.120201 «Pharmacy». In 2012, the order of the Ministry of Education and Science of Ukraine No. 1452 of December 20, 2012 approved the branch standard of higher education (BSHE) for the Bachelor of Pharmacy preparation in speciality 6.120201 «Pharmacy», which envisages obtaining basic higher pharmaceutical education in the IHE at the accreditation level II.

In order to bring the titles of pharmacy qualifications in line with the practices of most countries, draft amendments to the National Classifier of Occupations (DK 003:2010) and the Handbook of Qualification Characteristics of Occupations of Workers (Issue 78 «Health Care») have been prepared. In these draft of normative documents, it is proposed to replace the title of Provisor qualification for the Pharmacist and the title of Pharmacist qualification for the Assistant Pharmacist. These draft documents are now under public expert discussion. Given the above, the pharmacist is the professional who has a complete higher pharmaceutical education.

With the adoption of the new wording of the Law of Ukraine «On Higher Education», which introduced five degrees of higher education (Junior Bachelor, Bachelor, Master, Doctor of Philosophy, Doctor of Sciences), the Junior Specialist and Specialist degrees were abolished. The last

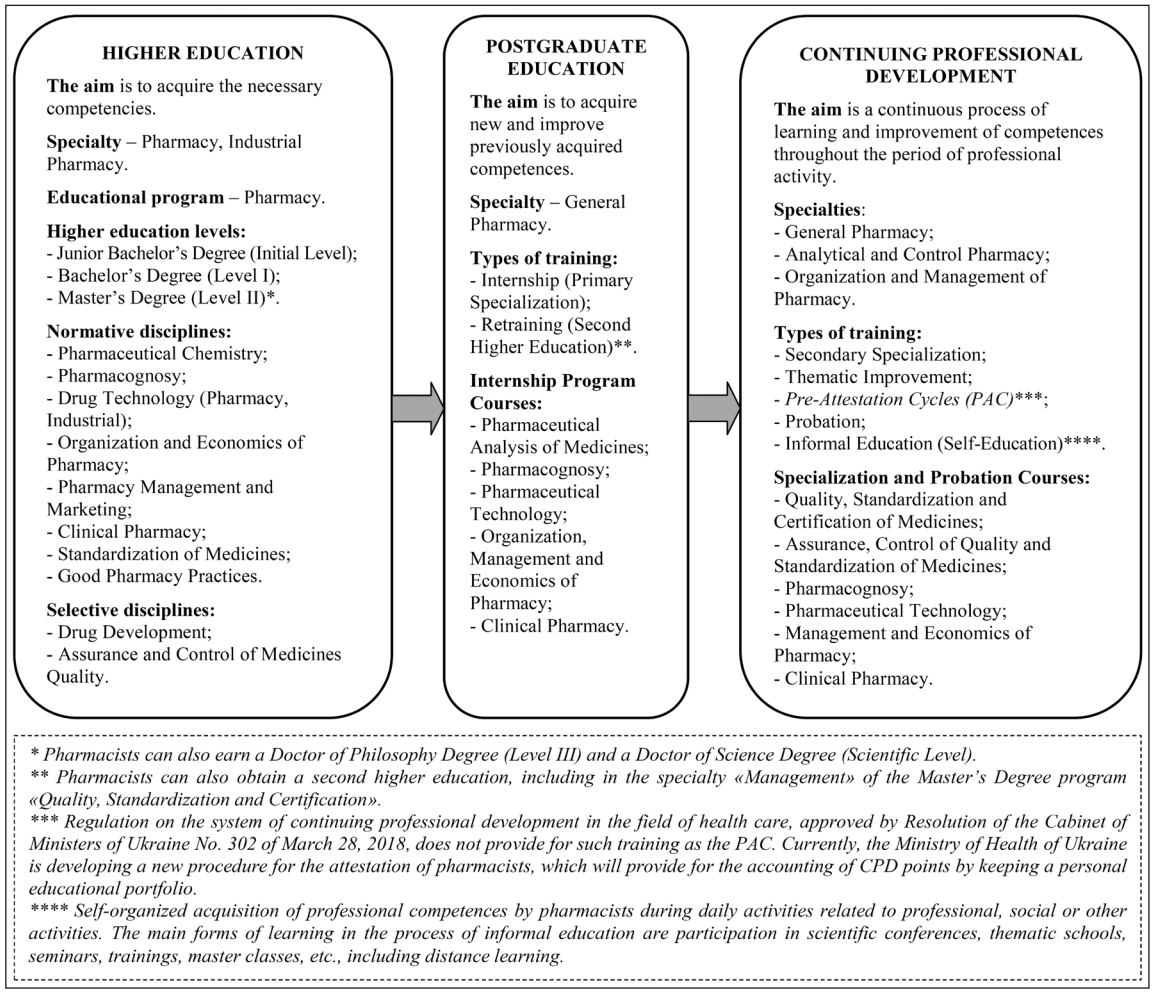

Fig. 1. The main stages of formation of professional competencies of pharmacists on issues of medicines quality assurance

admission to the Specialist Degree was held in 2016. After the entry of this Law into force Specialist, diplomas have been equated to Master degrees. The last admission to the Junior Specialist Degree was held in 2019. Junior Specialist diplomas have been equated to Junior Bachelor degrees.

As of 2018, the BSHE draft for the Master of Pharmacy preparation has been published on the official website of the Ministry of Education and Science of Ukraine. Based on the results of the expert discussion at the level of the professional community and the Scientific and Methodological Subcommittee on Pharmacy of the Ministry of Education and Science of Ukraine, it was decided that further preparation of Masters of Pharmacy would be end-to-end (without Bachelor Degree). That is, after the acceptance of the BSHE for the Master of Pharmacy preparation (planned in 2020), the preparation of Bachelors of Pharmacy will be stopped. Also, as of 2019, the BSHE draft for the Doctors of Philosophy in Pharmacy preparation has been published. The BSHE draft for the Junior Bachelor of Pharmacy preparation is planned to be published in 2020.

A separate branch of knowledge entitled 1202 «Pharmacy» existed in Ukraine until 2015. Resolution of the Cabinet of Ministers of Ukraine No. 266 of April 29, 2015 approved a new list of branches of knowledge and specialities for which higher education applicants are trained. This act, within the 22 «Health Care» branch of knowledge approved the 226 «Pharmacy, Industrial Pharmacy» speciality, which according to the BSHE draft for the Master of Pharmacy preparation includes two specializations: 226.01 «Pharmacy» and 226.02 «Industrial Pharmacy».

THE AIM

The aim of the study is to analyze, summarize and substantiate modern approaches to the formation of the professional competencies of pharmacists on issues of medicines quality assurance in Ukraine.

MATERIALS AND METHODS

Research methods are: systematic review, systematic and comparative analysis, content analysis, generalization, document analysis, logical and graphical modeling.

REVIEW AND DISCUSSION

We have studied and summarized the main stages of formation of professional competencies of pharmacists

Table I. The content of professional competences of the graduates of the Master of Pharmacy degree program, related to issues of assurance and control of medicines quality

Designa-tion	The brief content of competence that implies the ability to perform the following types of work
	1. Pharmaceutical competencies in the field of health care*
PC 1	To carry out sanitary-enlightenment work among the population
PC 2	To advise on the use of prescription and over-the-counter medicines, perform pharmaceutical care during the selection and realization of over-the-counter medicines
	2. Competence in the field of providing pharmaceutical assistance to the population
PC 4	To ensure rational use of prescription and over-the-counter medicines
PC 5	To monitor the effectiveness and safety of medicines use
PC 7	To ensure proper storage of medicines according to their physicochemical properties and Good Storage Practice (GSP) regulations in pharmacy institutions and hospitals
	3. Organizational and managerial competences
PC 8	To organize pharmacy activities in accordance with Good Pharmacy Practice (GPP) regulations and conduct commodity analysis
PC 10	To develop, implement and apply management approaches in the professional activity of pharmacy institutions and pharmaceutical enterprises, demonstrate leadership skills
	4. Professional and personal competences
PC 12	To use knowledge of legal acts and recommendations of good pharmacy practices in professional activities
PC 13	To demonstrate and use communication skills, principles of pharmaceutical ethics in accordance with the Code of Ethics for Pharmaceutical Workers of Ukraine, and WHO recommendations in practice activities
PC 14	To organize and carry out production activity of pharmacies for the manufacture of medicines in accordance with Good Pharmacy Practice (GPP) regulations
PC 15	To organize and participate in the production of medicines in the conditions of pharmaceutical enterprises in accordance with Good Manufacturing Practice (GMP) regulations, with appropriate development and registration of necessary documentation. To determine the stability of medicines
PC 16	To organize and carry out collection of medicinal herbal raw materials (MHRM) in accordance with Good Agricultural and Collecting Practice (GACP) regulations as a guarantee of quality of MHRM and medicines based on it
	5. Competencies in the field of quality management and quality control of medicines
PC 18	To develop and implement quality management systems for pharmaceutical enterprises and pharmacy institutions in accordance with standards, perform quality audits and manage risks for the medicines quality
PC 19	To organize and carry out medicines quality control in accordance with the requirements of State Pharmacopoeia of Ukraine and good pharmaceutical practices, determine the methods of sampling for the control of medicines and carry out their standardization in accordance with current requirements, prevent the spread of falsified medicines
PC 20	To develop methods of medicines quality control, including active pharmaceutical ingredients, medicinal plant raw materials and excipients using various control methods

*The division of special (professional) competences into five clusters is carried out in accordance with the Global Competence Framework for pharmacy specialists of the education initiative of International Pharmaceutical Federation (FIP), taking into account national peculiarities of preparation for higher pharmaceutical education applicants.

on issues of medicines quality assurance. The analysis have showed that the basic competences on these issues are acquired by students of IHE on speciality «Pharmacy, Industrial Pharmacy», while studying specialized pharmaceutical disciplines in senior courses (3–5 courses) [3]. Increasing the level of practical preparation, acquiring new and improving the previously acquired competencies of graduate pharmacists is carried out in the institutions of postgraduate pharmaceutical (medical) education (IPGE) at the stages of postgraduate education (PGE) and CPD (Figure 1). According to the Regulation on the system of continuing professional development in the field of health care, approved by Resolution of the Cabinet of Ministers of Ukraine No. 302 of March 28, 2018, the services for the CPD of pharmacists may also be provided by other providers of educational services (higher education institutions, research institutions, health care institutions, professional associations, etc.).

We have analyzed the list of competencies of the graduate with the Master of Pharmacy qualification, related to the issues of assurance and control of medicines quality and identified in the corresponding BSHE draft. The analysis

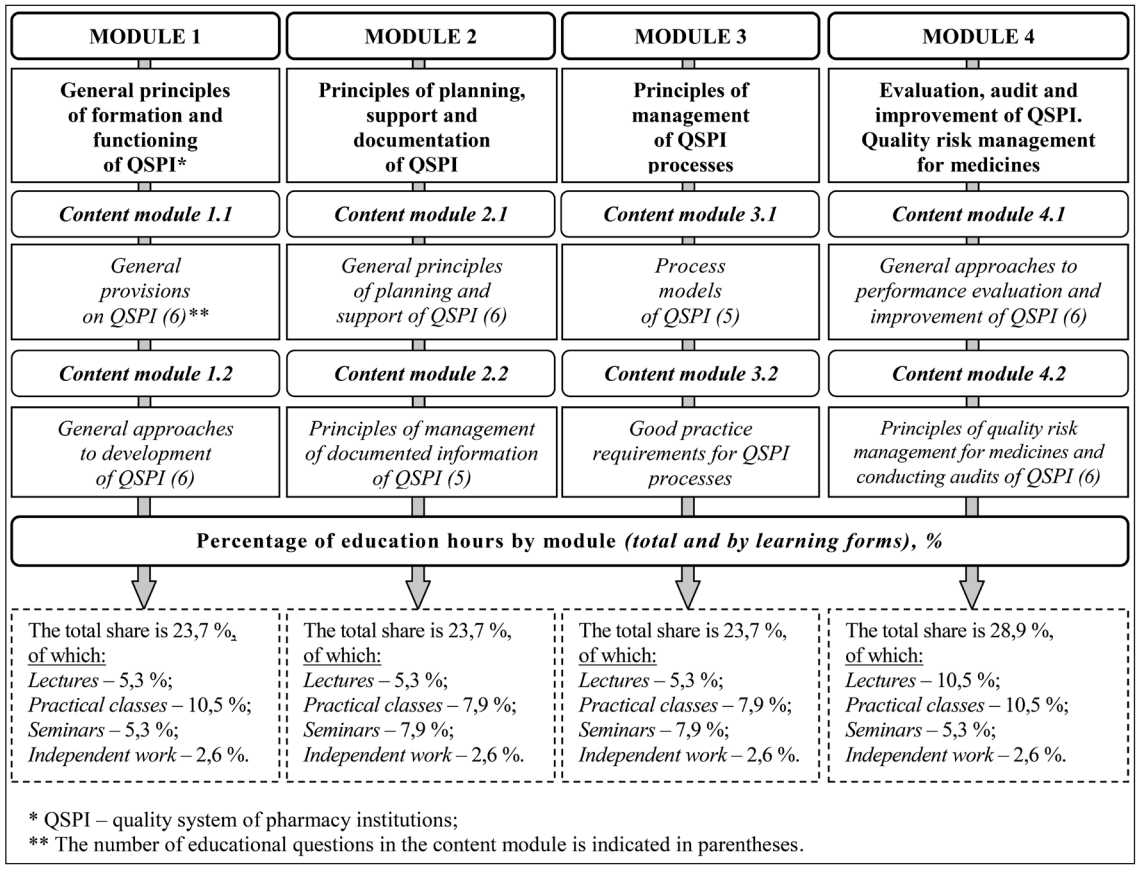

Fig. 2. The structure of curriculum and program of the «Basic foundations of the functioning of quality systems of pharmacy institutions» pharmacists' thematic improvement cycle

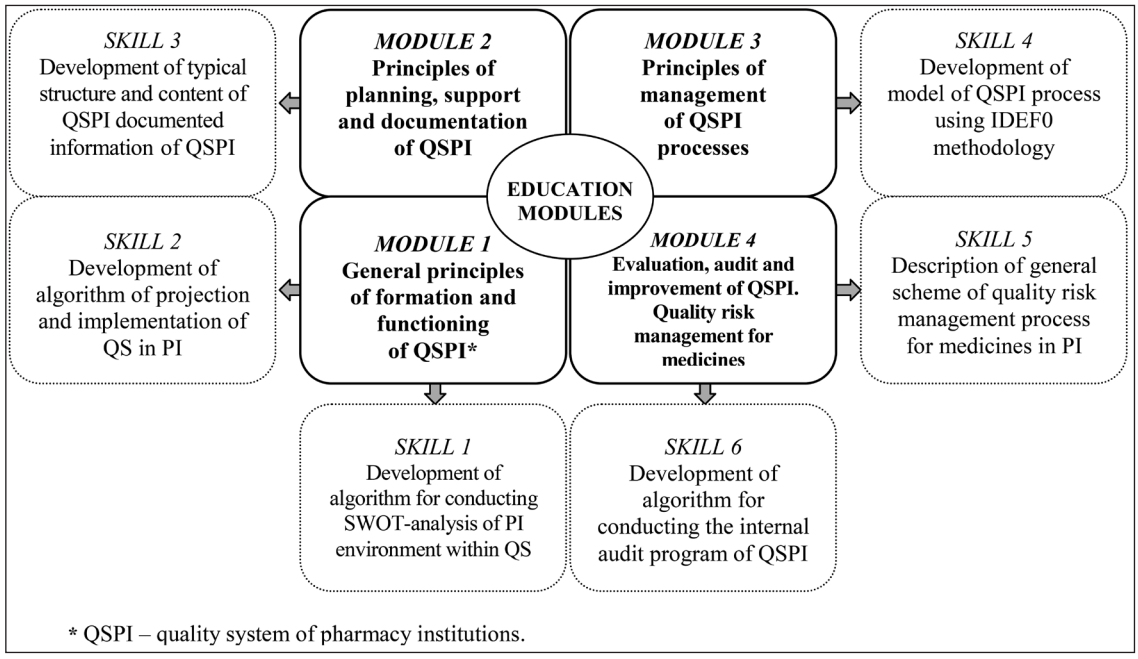

Fig. 3. The scheme of formation of practical skills within the «Basic foundations of the functioning of quality systems of pharmacy institutions» pharmacists' thematic improvement cycle

conducted showed that the content of the integral, 14 general and 15 of the 20 identified by the BSHE draft special (professional) competencies corresponds to the tasks facing the specialists who are responsible for assurance and control of medicines quality at the stages of production, wholesale and retail realization. The analysis of the program learning outcomes identified in the BSHE draft for the Master of Pharmacy preparation shows that

they are fully consistent with the content of competencies described in the Table I.

In the context of studying the current state of preparation of pharmacy specialists at the stages of PGE and CPD, the educational activity of the Department of Quality Control and Standardization of Medicines of Shupyk National Medical Academy of Postgraduate Education has been analyzed. So, in recent years the department has been teaching the main issues of medicines quality assurance on cycles of internship or primary specialization (for persons who obtain the specialist certificate in speciality «General Pharmacy»), secondary specialization (for persons who obtain the specialist certificate in speciality «Analytical and Control Pharmacy»), probation (cycles for persons who confirm the Pharmacy specialist certificate in relevant specialty) and pre-attestation preparation (cycles for persons applying for assignment of the Pharmacist Qualification Category in relevant speciality) within the courses «Pharmaceutical Analysis of Medicines», «Quality, Standardization and Certification of Medicines», «Assurance, Control of Quality and Standardization of Medicines», «Pharmacognosy».

The most topical aspects of medicines quality assurance are taught at the thematic improvement cycles (TIC), whose programs are constantly updated. So, during 2014–2018, the department conducted the «Topical issues of quality assurance and prevention of the spread of falsified medicines» TIC for pharmacists-analysts, chemists-analysts, heads of pharmacy institutions and Responsible persons in medicines quality. At present, much of the issues previously taught at these cycles are included in the Internship program, as well as the educational programs «Pharmacy» that are taught in IHE and provide for the study by students of 5th year course of new narrow normative and selective disciplines on medicines quality assurance («Standardization of Medicines», «Good Pharmacy Practices», «Assurance and Control of Medicines Quality», etc.). Given this, it becomes obvious the need for the development and implementation of the short-term TIC with elements of distance learning in the educational process, which would provide the formation of a high level of professional competence of pharmacy professionals, in particular on the implementation of effective quality systems in pharmacy institutions [4].

In the context of the above mentioned, we have been worked out the curriculum and program of the «Basic foundations of the functioning of quality systems in pharmacy institutions» thematic improvement cycle for pharmacists. The aim of this cycle is to acquire professional competencies in the development, implementation and support of effective functioning of quality systems in pharmacy institutions (pharmacy warehouses, manufacturing and retail pharmacies, pharmacy chains) and hospital pharmacy services [5, 6]. The contingent of this TIC may involve the pharmacists of different specialities, including those who perform the functions of Responsible persons in medicines quality, hold the positions of heads of pharmacy institutions, quality services of wholesale and retail pharmaceutical enterprises, hospital pharmacy services, specialists of the State Service of Ukraine on Medicines and Drugs Control, and scientific-pedagogical workers of IHE and IPGE, which provide training to pharmacy specialists at the stage of PGE and CPD. The TIC program includes four education modules, each of which contains two content modules, and provides for the study of the following issues: general principles of formation and functioning of quality system of pharmacy institutions (QSPI); principles of planning, support and documentation of QSPI; principles of management of QSPI processes; general approaches to monitoring, evaluation, audit and improvement of QSPI; quality risk management for medicines [7, 8, 9]. In addition to the lecture classes, the program also envisages time for extracurricular independent work without the participation of the lecturer. Education hours along the forms of training include: lectures – 26,3 %, practical classes – 36,9 %, seminars – 26,3 %, independent work – 10,5 % of the total number of academic hours, respectively. The structure of curriculum and program of the TIC is presented in the Figure 2.

The TIC program, along with the study of different aspects of the implementation and functioning of quality systems in pharmacy institutions, also envisages study issues of the formation of quality systems in hospital pharmacy services, which is part of the entire quality management system (QMS) of hospitals. In total, the TIC program contains 46 study questions, on the basis of which education topics are formed and a thematic plan is developed.

Forms of control of the educational process include the assessment of the level of mastering practical skills and oral test. In the framework of our work, a list of theoretical questions to the test and standards of practical skills have been developed. At the same time, the list of practical skills developed covers all four modules of the TIC program (Figure 3).

The duration of training on the cycle is 0,5 months (78 education hours) and in shortened form – 0,25 months (39 education hours). The organization of training can be conducted in full-time and full-time/part-time forms with the elements of distance learning. The share of distance hours in distance learning is about 50 % of the total academic hours within the cycle. Distance learning involves online and offline communication, using email, video, and online learning resources.

Thus, the implementation of short-term TIC with the elements of distance learning at the CPD stage is one of the most effective forms of improving the professional competence of pharmacy specialists on the issues of QSPI functioning. The content of the education material of TIC program developed by us makes it possible to prepare pharmacists for independent work in the field of implementation and support of effective QS in pharmacy institutions and hospital pharmacy services. The further perspective is the development of highly specialized one-, two-day programs for intensive training of pharmacists on the most topical issues of functioning of QSPI, which involves the use of current forms of training (seminars,

trainings, webinars, master classes, etc.) and active use of video resources.

In the context of the substantiation of modern approaches to the training of pharmacy specialists on issues of medicines quality assurance, we have studied the experience and benefits of using modern forms of training at the stages of PGE and CPD of pharmacists. The modern approaches to the preparation of pharmacists require the implementation of new forms of training in the educational process, the development of the most favorable conditions for the acquisition of program material, the formation of skills and abilities. One of the way to the optimization of training on the pharmacists' refresher cycles is to use training bases that are selected according to the requirements of curriculum and program. Many years of experience of the department show that conducting classes on training bases increases the interest of listeners to the studies, allows to present the education material in a most qualitative, clear and comprehensive way, which is not always possible in the lecture class. It creates the opportunity to involve highly qualified practitioner specialists without the interruption of work for teaching, and promotes the use of new forms and methods of training.

So, the department actively implements different modern forms of seminars, such as: scientific and practical seminar, Flash seminar, blitz seminar, seminars in the form of conversation, debate, conference, consultation, presentation, excursion, exhibition, round table, colloquium, practical work, training, master class, situational games, etc. At the classes held at the training bases, due attention is paid to the issues of medicines quality assurance, in particular the activities of Responsible persons on medicines quality, the methodology of conducting input control of medicines in pharmacy institutions and hospitals, mastering current methods of pharmaceutical analysis, prospects for the development and standardization of medicines, including herbal medicines and dietary supplements [3, 5].

It should also be noted that blended learning has been rapidly developing in the system of PGE and CPD for medical and pharmaceutical professionals, which involve the combination of distance and e-learning with traditional forms, such as full-time and part-time studies. The implementation of blended learning methods provides for the intensification, modernization of education process and increases its efficiency. The blended learning model assumes that part of the learning activity is spent by the listener in the classroom, and part is taken to a distance form, dominated by independent types of work. At the same time, it is important for the teacher to determine what types of listener activities should be used in face-to-face classes, and what types can be transferred to an independent distance form. Thus, the face-to-face form provides listeners with new material to familiarize themselves with the most difficult problems that require the direct involvement of the teacher. In the classroom, the teacher conducts discussions, work in groups, that is, activities that require direct contact at different levels (with the teacher, between the listeners, etc.). Distance learning includes independent research activities, practical work and group assignments, consultations with the teacher, conducting tests.

The department has accumulated considerable experience in the use of blended learning forms during the conduct of cycles on thematic improvement, specialization, probation, pre-attestation preparation in specialities «Analytical and Control Pharmacy» and «General Pharmacy». The first stage of implementation of blended learning methods at the department has been characterized by an increase in the share of teachers with extensive experience in practical work, optimization of the «listener – teacher» ratio (1:5), expansion of the register of training bases, active use of computer programs for testing and presentations (mainly in PowerPoint). At the second stage, the resources of local and global networks, e-learning tools (teaching materials, manuals, textbooks, electronic versions of pharmacopoeia, etc.) have been put to use. The modern forms of seminars and elements of online learning through the Internet have been implemented. At present, the department is on the threshold of the third stage, which is related to the development of different models of learning management and approaches to the evaluation of its quality and effectiveness, the development of software systems that provide a comprehensive solution to the problems of blended learning (learning content management systems, systems of testing and monitoring of learning outcomes, interactive support systems of learning environment and learning management systems). It is possible to implement this stage only if the high level of competence of the teachers of the department will be ensured in the following areas: the development of high-quality electronic educational and methodological complex for the discipline; scenario development of study of disciplines in the blended form; providing pedagogical support for the process of study disciplines by listeners on the individual educational trajectory (tutorial support); evaluation of learning outcomes of listeners and systematic monitoring of the learning process quality in the blended form to correct the elements of the system of professional training of specialists; providing educational communications by means of email, audio and video. Thus, the implementation of the blended learning model at the stages of PGE and CPD increases the efficiency of professional training of pharmacists, both interns and listeners of refresher courses.

CONCLUSIONS

The current state and content of preparation have been analyzed and main stages of formation of professional competencies of pharmacists on issues of quality assurance and quality control of medicines at the stages of obtaining higher education, PGE and CPD have been summarized.

The curriculum and program of the «Basic foundations of the functioning of quality systems in pharmacy institutions» thematic improvement cycle for pharmacists have been substantiated and developed. The content of the program provides an opportunity to prepare pharmacists for independent work in the field of implementation and

support of effective quality systems in pharmacy institutions and hospital pharmacy services.

In the context of substantiation of modern approaches to the preparation of pharmacists in the field of medicines quality assurance, the experience and advantages of such modern forms of training of pharmacists as the use of training bases and blended learning have been studied.

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REVIEW ARTICLE

MODERN CONCEPT OF UNDERSTANDING THE HUMAN RIGHT TO LIFE

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ABSTRACT

The aim: The purpose of this article is to expose the essence of the concept of the human right to life, including in the content of this right, the duty of the state to maintain and develop general conditions for a dignified human life.

Materials and methods: The article explores the modern concept of understanding the human right to life. The article analyzes the constitutions, other regulations, as well as the experience of different countries in the world on this issue (in particular, the countries of Western and Eastern Europe, Latin America, USA). The empirical basis of this research consists of two judgments of the Constitutional Court of Ukraine, Resolution of the Plenum of the Supreme Court of Ukraine «On Judicial Practice in Cases of Crimes against the Life and Health of a Person» of February 7, 2003, № 2, and judgments of the European Court of Human Rights (Case of Lambert and others v. France of 5 June 2015, Case Hristozov and others v. Bulgaria of 13 November 2012, Case G. N. and others v. Italy of 01 December 2009) on issues related to the human right to life. The application of methods and techniques of scientific knowledge is conditioned by a systematic approach, which enables them to consider outlined problems in the unity of their social content and legal form. In particular, the formal-logical method, methods of analysis and synthesis, comparative-legal method, formal legal and statistical methods are used.

Conclusions: The modern approach to understanding the human right to life presupposes that it is the state's responsibility to protect that right, to take appropriate measures to remedy the general conditions in society that may endanger life or prevent individuals from living a dignified life.

KEY WORDS: human rights, right to life, death penalty, suicide, euthanasia, abortion, medical error, worthy life

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INTRODUCTION

Much of the democratic states of the world have enshrined at the constitutional level the provisions guaranteeing everyone the right to life (for example, Article 20 of the Constitution of the Russian Federation, amendment V to the US Constitution, Article 5 of the Constitution of Brazil, Article 31 of the Constitution of Japan, Article 15 of the Constitution of Spain, 2 of Article 2 of the Constitution of Germany, Article 38 of the Constitution of Poland, Article 24 of the Constitution of Moldova, Article 27 of the Constitution of Ukraine, etc.).

The human right to life is a fundamental, inalienable right of everyone, which, unfortunately, is often violated, and we, therefore, consider it worthwhile to pay close attention to it. The relevance of this issue is enhanced by the fact that exploring the right to life, we can distinguish another right – the right to a worthy life, which requires scientific analysis. It should be noted that the human right to life has become the subject of many works of scientists, but the mentioned problems do not lose their relevance and need further research.

THE AIM

The aim of this article is to reveal the essence of the concept of «human right to life», to analyze its constituent elements; to investigate international human rights instruments concerning the right to life, and to consolidate this right in the constitutions of different countries of the world; consider human rights issues (death penalty, suicide, euthanasia, abortion, medical error) in the light of experience from around the world, as well as the concepts of «necessary defense» and «extreme necessity». It also seeks to highlight the possibility of including in the human right to life the duty of the state to maintain and develop general conditions for a worthy human life.

MATERIALS AND METHODS

The article explores the modern concept of understanding human right to life. The article analyzes the constitutions, other regulations, as well as the experience of different countries in the world on this issue (in particular, the countries of Western and Eastern Europe, Latin America, USA). The empirical basis of this research consists of two judgments of the Constitutional Court of Ukraine, Resolution of the Plenum of the Supreme Court of Ukraine «On Judicial Practice in Cases of Crimes against the Life and Health of a Person» of February 7, 2003, № 2, and judgments of the European Court of Human Rights (Case

of Lambert and others v. France of 5 June 2015, Case Hristozov and others v. Bulgaria of 13 November 2012, Case G. N. and others v. Italy of 01 December 2009) on issues related to the human right to life.

The methodological basis of the study is the methods and techniques of scientific knowledge. Their application is conditioned by a systematic approach, which enables them to consider outlined problems in the unity of their social content and legal form. In particular, the formal-logical method was applied to analyze the elements of the human right to life, as well as to establish the essence of the concept of the human right to life and other related concepts. Methods of analysis and synthesis have revealed the logical structure of the concept of human rights to life, the construction of definitions and other theoretical constructs, and the comparative-legal method – to compare the laws of different countries of the world concerning the issue of human rights to life. To formulate the concepts of «human right to life», «suicide», «euthanasia», «abortion», etc., a formal legal method was used. The statistical method has helped to investigate statistical information on abortion worldwide.

REVIEW AND DISCUSSION

The primary in origin and meaning and inalienable human right is its right to life that arises from birth. This right cannot be revoked or restricted by anyone.

It is interesting that in many countries the law provides that the right to a person's life arises from its conception. In particular, in Japan, a person's age is calculated from the time of conception. The term «nasciturus» comes from Latin and means the one who is to be born, a child who is still inside his/her mother's womb. From the moment of conception until his/her birth, a child inside his/her mother's

womb undergoes various stages, and nasciturus would be a general term for a child inside his/her mother, until the moment of his/her birth or at least the beginning of childbirth [1]. The nascitrus has a number of personal and property rights, protected mainly by private law.

The right to life is an inalienable, fundamental human right that relates to civil (personal) rights in the system of fundamental rights and freedoms of person and citizen. By the way, this right is a natural human right, it is enshrined in the most important international human rights instruments.

Thus, Article 3 of the Universal Declaration of Human Rights of 10 December 1948 states that everyone has the right to life, liberty, and security of person [2].

Article 6 of the International Covenant on Civil and Political Rights of 16 December 1966 provides that every human being has the inherent right to life. This right shall be protected by law. No one shall be arbitrarily deprived of his life. In countries which have not abolished the death penalty, sentence of death may be imposed only for the most serious crimes in accordance with the law in force at the time of the commission of the crime and not contrary to the provisions of the present Covenant and to the Convention on the Prevention and Punishment of the Crime of Genocide. This penalty can only be carried out pursuant to a final judgment rendered by a competent court. When deprivation of life constitutes the crime of genocide, it is understood that nothing in this article shall authorize any State Party to the present Covenant to derogate in any way from any obligation assumed under the provisions of the Convention on the Prevention and Punishment of the Crime of Genocide. Anyone sentenced to death shall have the right to seek pardon or commutation of the sentence. Amnesty, pardon or commutation of the sentence of death may be granted in all cases. Sentence of death shall not be imposed for crimes committed by persons below eighteen years of age and shall not be carried out on pregnant women. Nothing in this article shall be invoked to delay or to prevent the abolition of capital punishment by any State Party to the present Covenant [3].

Article 2 of the Convention for the Protection of Human Rights and Fundamental Freedoms of 4 November 1950 provides that everyone's right to life shall be protected by law. No one shall be deprived of his life intentionally save in the execution of a sentence of a court following his conviction of a crime for which this penalty is provided by law. Deprivation of life shall not be regarded as inflicted in contravention of this Article when it results from the use of force which is no more than absolutely necessary: (a) in defence of any person from unlawful violence; (b) in order to effect a lawful arrest or to prevent the escape of a person lawfully detained; (c) in action lawfully taken for the purpose of quelling a riot or insurrection [4].

For the first time at the legislative level, the right to life was enshrined in the text of the Declaration of Independence of the United States in 1776.

The provisions of these international legal acts have been reflected in many constitutions of the countries of the world. For example, in Art. 15 of the Spanish Constitution states that «everyone has the right to life, to physical and moral integrity, and no one can, in any case, be subjected to torture or to inhuman or degrading treatment» [5]. Art. 38 of the Constitution of Poland enshrined the provision that the Polish Republic guarantees every person legal protection of life [6]. In Art. 5 of the Brazilian Constitution states that Brazilians and foreign residents have the right to life, liberty, equality, security and property [7]. Amendment V (1791) to the US Constitution provides that no person shall be nor be deprived of life, liberty, or property, without due process of law [8]. Understanding of the right to life under the Constitution of Ukraine is possible only in the context of at least two of its articles – 3 and 27, where Article 3 forms the fundamental value approach of the Ukrainian state to a person, his life and health as to the «highest social value» in Ukraine, and in Article 27 sets out the general content of the personal right to life: «Everyone has the inherent right to life. No one can be arbitrarily deprived of his life. It is the duty of the state to protect human life. Everyone has the right to defend his life and the health, life, and health of others against unlawful encroachment» [9].

The current understanding of the right to life implies the abolition of the death penalty. According to international experience, the death penalty has not been justified as an effective tool in the fight against crime. This punishment does not apply to crime-deterrents. That is why around 100 countries have abolished the death penalty (Australia, Brazil, almost all European countries). However, in some countries, the death penalty continues to persist (China, Iran, Iraq, Saudi Arabia, North Korea, etc.).

In July 2019, the US Department of Justice decided to return to the death penalty. For the past 16 years, the unofficial moratorium has been at the highest level at the federal level. The last federal government in the United States applied the death penalty in 2003. At the state level, this punishment could continue to be applied. In the United States, the death penalty is administered by administering to prisoners an injection of pentobarbital (a drug that gradually slows down the body, including the nervous system, and eventually leads to death) [10].

Structurally, the right to life can be considered as having three components: 1) the inalienability of the human right to life; 2) prohibition of arbitrary deprivation of life; 3) the right to protect one's life and the lives of others against unlawful encroachment. As noted above, the right of a person to deprive another person's life as a result of the inevitable need to use force in cases determined by law is recognized [11, p. 186].

The inherent right to life belongs to everyone regardless of race, color, political beliefs, citizenship, etc. The inalienability of the right to life should be seen as a consequence of the naturalness of that right. No one, including the Constitution, gives a person the right to live.

To better understand the essence of the human right to life, let us turn to the national judicial practice of Ukraine.

It should be noted that according to the Judgment of the Constitutional Court of Ukraine in the case on the constitutional petition of 51 people's deputies of Ukraine on the constitutionality of the provisions of Articles 24, 58, 59, 60, 93, 190-1 of the Criminal Code of Ukraine in part, which provides for the death penalty as a form of punishment (Death Penalty Case) of 29 December 1999 № 11-rp/1999, there is every reason to believe that the inalienable right of every person to life is inextricably linked with his right to human dignity. As basic human rights, they determine the possibility of exercising all other human rights and freedoms and can be neither restricted nor abolished [12].

It is the duty to protect a person's life and, therefore, to guarantee the right to a life that is constituted, above all, by the state. The state itself assumes certain obligations to protect human life. Such duties should include, first of all, the following: establishing legal (criminal) responsibility for the unlawful encroachment on a person's life and the unlawful deprivation of his or her life; the prohibition to deprive any person of his life arbitrarily; prohibition of extradition of a person to a state in which the death penalty may be applied; a ban on the expulsion of a person to a country where his or her life is threatened; introduction of legal remedies for protection of the right to life, in particular when there is a high likelihood of absolute threat to human life; introducing legal safeguards to protect a person who protects his or her life or health or the lives and health of others from unlawful encroachment [11, p. 186–187].

The Constitutional Court of Ukraine considers that the positive obligation of the state to implement an adequate system of protection of life, health, and human dignity is to ensure effective investigation of the facts of deprivation of life and ill-treatment, including persons in places of imprisonment under state control [13].

There are a number of legal acts in the laws of different countries of the world aimed at ensuring the human right to life. In particular, these are criminal codes (criminal responsibility for intentionally or negligently depriving a person of his or her life; introducing concepts of necessary defense and extreme necessity).

Those States which have ratified the Convention for the Protection of Human Rights and Fundamental Freedoms are not entitled to extradite a person to States in which the death penalty may be applied.

The duty of the state to ensure the right to life and thus to protect the life of a person should be regarded as both its positive and negative constitutional obligation [11, p. 187]. A positive obligation is to oblige state bodies (in particular, the legislature and other state bodies empowered to adopt by-laws) to enforce a constitutional prescription for the creation of legislation that would ensure the right to life. Such activity is absolutely necessary in view of the complexity of this right and the development in the world of ideas about its content. This activity also applies to the full national implementation of acts of dynamic interpretation by the international bodies of ratified international human rights treaties [11, p. 187–188]. The state's negative obligation on the human right to life is the obligation to create the appropriate organizational, personnel, financial and other conditions to ensure the implementation of the relevant legislation. The inability or unwillingness of the state to do so will be indicative of a violation of the constitutionality of the state, for example, a brazen delay in carrying out the necessary investigative measures in a murder case or the refusal of judges to apply the European Court of Human Rights (hereinafter – ECHR) decisions on the protection of the right to life when this is the basis [11, p. 188].

According to the Resolution of the Plenum of the Supreme Court of Ukraine of February 7, 2003, № 2 «On Judicial Practice in Cases of Crimes against the Life and Health of a Person», the courts of Ukraine generally comply with the requirements of the legislation regulates liability for crimes against life and health of a person.

However, there are cases when during the trial of this case categories are allowed violations of both material and procedural law.

The Court ruled that one of the important guarantees of the implementation – the human right to life and health which is proclaimed by articles 3 and 27 of the Constitution of Ukraine, is unconditional fulfillment by courts of requirements of criminal procedure the law on ensuring the rights of victims of these crimes.

In cases of crimes of this type, the courts are obliged as to establish the guilt of the defendants and assign them the necessary and sufficient to correct them and prevent new crimes of punishment, and take all necessary measures until full reimbursement material and moral damage caused to the victim. According to Part 1 of Art. 64 of the Criminal Code of Ukraine, life imprisonment is appointed only in cases specifically provided for therein Code, and provided that the court does not consider it possible to apply imprisonment for a definite term. The purpose of this punishment must be motivated in the sentence with the obligatory indication of circumstances, which, according to the court, prevent the application of imprisonment on a certain period. If several are found guilty of a crime persons sentenced to life imprisonment shall be sentenced the relevant motives for each of them shall be stated separately [14].

The right to life does not belong to the absolute, that is, there is no absolute, unrestricted prohibition on depriving a person of life. In some cases, a person may be deprived of life because of the inevitable need to use force. But such cases, since they limit human rights, must be determined solely by the laws of the state. In addition, as noted above, international human rights instruments (for example, Art. 2 of the Convention for the Protection of Human Rights and Fundamental Freedoms) refer to cases of the extreme necessity to use force [11, p. 188].

The laws of the states may establish the grounds for the use of coercive measures, in particular, firearms (for example, Art. 17 of the Law of Ukraine «On the National Police»). The ECHR and other international human rights bodies consider proportionality, expediency and absolute necessity as the main criteria in the legal assessment of the use of force, including weapons, in the course of a lawful arrest or the prevention of the escape of a lawfully detained person. Thus, according to the legislation of Ukraine, it is forbidden to use police force, special means and firearms for women with obvious signs of pregnancy, minors, persons with obvious signs of disability or old age, except in cases of armed or group attack, armed resistance to police that threatens the lives and health of others or police officers if it is not possible to repel such an attack or resistance by other means and means. The police are also forbidden to use firearms in places where harm can be caused to other persons, as well as inflammable and explosive places, except in cases of necessity to repel an attack or an extreme necessity (Part 5 of Art. 43, Part 9 of Art. 46 of the Law of Ukraine «On the National Police») [15].

All international legal practice concerning the assessment of the legitimacy of deprivation of life when committing lawful acts to suppress an uprising or rebellion is based on the application of the principle of «absolute necessity» [11, p. 190].

In exploring the human right to life, one cannot escape the contemporary problematic issues associated with this right, such as suicide, euthanasia, and abortion.

Suicide is the deprivation of one's self (without assistance) of one's physical life, which occurs as a result of a voluntary, deliberate decision, or as a result of affect.

Suicide is an extreme form of a wide range of self-destructive behavior. The latter is considered to include various indirect forms of behavior, such as intentional and deliberate harm to life-threatening and threatening, self-harm (some extreme sports, risky behavior). The most dramatic form of suicidal behavior is so-called «prolonged suicide», when a person, taking his own life, decides to «withdraw» people from the environment. Fortunately, these situations are very rare, often the result of extremely severely altered thinking (for example, a mother is convinced of a serious illness of a child, suffering, unhappiness, catastrophe; she believes that death is the best solution and help for him). Fortunately, suicide attempts usually do not end in death. They are an expression of human helplessness to life's problems, a cry for help – often unknown. According to statistics, women are much more likely to attempt suicide, men are more likely to actually save their own lives. The choice of method plays a role here. For example, men in Poland usually try to hang themselves (in the US – they use firearms), while women try to take a large number of drugs. Often, these are sleeping pills and tranquilizers (according to the popular belief that «you can fall asleep after them and not wake up») that are actually relatively toxic. It should be noted that suicide should not be considered a consequence of mental disorders. One can imagine a mentally healthy person committing suicide (for example, for ideological reasons, suicide during the war, after the arrest, fearing that torture will cause others to fall, or the suicide of a person who knows about a terminal illness and wants to avoid suffering, related). In the latter case, the decision on suicide is quite similar to the decision on euthanasia. Of course, a mentally healthy person has the psychological ability to make the decision to end their life at the «optimal» moment for themselves. On the other hand, the vast majority, as many as 80% of suicide victims, are people with mental disorders [16].

The modern theory of law proceeds from the general recognition of the right of the person to consciously dispose of his own life. In the case of a mental disorder of a person who commits or manifests a real intention to take actions that present an immediate danger to him or others or is unable to satisfy his or her basic needs independently at a level that ensures his or her vital activity, such person may be involuntarily admitted to a psychiatric care facility. It should be noted that driving a person into suicide is a crime (Art. 120 of the Criminal Code of Ukraine, Art. 151 of the Criminal Code of the Republic of Poland, etc.).

Euthanasia (Greek ευ – good + Greek. θάνατος – death) should be distinguished from suicide – the artificial deprivation of a person's life in the presence of will on his part by medical means in cases of incurable illness or serious health impairment (fatal injuries, etc.) of that person [11, p. 190]. According to such a criterion, as a method of implementation, euthanasia is divided into an active (positive or «filled syringe method»), that is the use of special means or other actions that result in a quick and painless death, and a passive (negative or «method of the deposited syringe»), which means the abandonment of measures conducive to

maintaining life, that is the termination of the provision of life-saving medical care that accelerates the ting natural death. According to another criterion, the subject of the expression, euthanasia is divided into voluntary, that is, the use of medicinal or other means to an incurable patient, which leads to a mild and calm death upon the request of a patient who is aware of his actions and can control them and compulsory, which means causing light death by means of appropriate means and actions in the incurable patient, but by the decision of the family members, legal representatives or public institutions [17, p. 31].

In states that recognize euthanasia, particular attention is paid to the legal regulation of establishing the will of a person who agrees to euthanasia. In international law, the question remains whether the right to life also covers a person's obligation to live and whether a person, with the help of another person, can knowingly relinquish that right. The practice of international human rights bodies leaves this issue at the discretion of each state [11, p. 190].

Nowadays the euthanasia is legal in the Netherlands, Belgium, Ireland, Colombia, and Luxembourg. Assisted suicide is allowed in Switzerland, Germany, Japan, Albania, Canada, and several US states. Most clearly, the right to euthanasia is formulated in the Netherlands legislation and in Belgian and Swiss, there are clearly formulated and consistent wishes of the patient to die [17, p. 29–30].

Thus, the Netherlands is the first country that legalized euthanasia. In 1982, in this country was established the Euthanasia Commission, and on April 10, 2001, passed the Law on the Control of the End of Life at Will and Suicide Assistance. While the Netherlands still had age restrictions for children (12 to 16 years old), in 2002, Belgium passed a law that allowed euthanasia without age restrictions. Euthanasia for foreigners certified by a doctor is allowed in Switzerland. In November 2015, Germany also adopted a law on euthanasia [18].

Euthanasia does not mean a painless and swift death, because in only 16 out of 100 cases people died without agony, and the rest died from prolonged and greater suffering [18].

However, if the law of the state does not in any circumstances recognize the validity of euthanasia, then it (euthanasia) is recognized as a crime.

On June 5th, 2015, the ECHR delivered its judgment in the case of Lambert and Others v. France. The case was about end-of-life decision-making on behalf of a persistently incompetent patient (Vincent Lambert, a French citizen) who was in a vegetative state and had to be artificially fed and hydrated through a gastric tube. The controversy arose with respect to the removal of that tube, which would result in the patient's starvation, dehydration, and, ultimately, death: while some of the patients' relatives (parents, half-brother, and sister) wanted him to be kept fed and hydrated, his other relatives (wife and nephew) and caring physicians wanted the nutrition and hydration to be discontinued.

The controversy was litigated in the French courts, including the Administrative Court and the Conseil d'État. The courts came to drastically different conclusions: while the Administrative Court opined that the decision to withdraw artificial nutrition and hydration from Mr. Lambert «had constituted a serious and manifestly unlawful breach of [his] right to life» the Conseil d'État held instead that the provision of the French Public Health Code authorizing physicians to withdraw and withhold «unreasonably obstina[te]» medical treatment «cannot be said to be incompatible with the requirements of Article 2 of the Convention [for the Protection of Human Rights and Fundamental Freedoms] …, or with those of Article 8 …». The Conseil d'État stressed that the law allowing the discontinuation of medical treatment provides for several procedural safeguards (reports about patient's medical condition, ascertaining his or her wishes about being kept alive while in a persistently unconscious state, consultations with patient's family members) and therefore meets the requirements of the Convention.

The ECHR, deciding on the application following the judgment of the Conseil d'État, focused its analysis on Article 2 of the Convention. In particular, the ECHR noted that the duty to protect human life, enshrined in Article 2, consists of both positive and negative obligations of the States (that is, the obligations to «take appropriate steps to safeguard the lives of those within [the] jurisdiction [of the State]» and the obligations to «refrain from the «intentional» taking of life».

With respect to negative obligations, the ECHR observed that the «therapeutic abstention» (that is, withdrawal and withholding of medical treatment) lacks the intention to end patient's life – by contrast, a doctor discontinuing medical treatment from his or her patient merely intends to «allow death to resume its natural course and to relieve suffering» [19; 20].

So, as to the judicial remedies that had been available to the applicants, the Court reached the conclusion that the present case had been the subject of an in-depth examination in the course of which all points of view could be expressed and that all aspects had been carefully considered, in the light of both a detailed expert medical report and general observations from the highest-ranking medical and ethical bodies. The Court concluded that the domestic authorities had complied with their positive obligations flowing from Article 2 of the Convention, in view of the margin of appreciation left to them in the present case, and that there would be no violation of Article 2 of the Convention in the event of implementation of the Conseil d'État judgment of 24 June 2014 [19].

The story of Mr. Lambert very much resembles the tragedy of Terri Schiavo in the United States, who was in a persistent vegetative state for more than ten years and was also artificially fed and hydrated. Her family also split with regard to her future: while the husband of Ms. Schiavo wanted the nutrition and hydration to be discontinued, her parents objected and wanted their daughter to be kept alive. The case arose a nation-wide controversy in the United States, culminating in a narrowly tailored bill approved by the Congress and signed by President Bush, and was ultimately decided in a federal court that approved the removal of the feeding tube [20].

On the mind of the teacher of bioethics, senior nurse of the palliative department in the Hospital of Metropolitan Andrey Sheptytsky Josaphat Drobik, from the point of view of moral theology of life is a gift of God and only He is the master of life and death. This is emphasized not only in Christianity but also in other religions. She noted that the more advances in euthanasia, the more the society departs from the moral standards of recognizing human life as the highest value on earth. It's like a slippery precarious slope on which we stand. But we must always remember that the question is not how we die, but how we live [18].

Abortion is an artificial abortion. There are debates and controversies around the world about the moral and legal status of abortion (the abortion controversy). The two main discussion groups call themselves «for choice» (with a clampdown on the right of women to choose) and «for life» (with a clampdown on the right of an unborn child to live). Each group, with different results, seeks to influence public opinion and seek legal support for its position. In some cases, the controversy was waged using violence. The legality of abortion varies from one country to another. For example, in Canada, abortions are available at the request of a pregnant woman, while abortions are prohibited in Ireland.

Globally, 25% of pregnancies ended in abortion in 2010–2014, meaning 56 million induced abortions each year during this period. Between 1994 and 2014, the abortion rate declined markedly in developed regions, from 46 to 27 per 1000 women of childbearing age. In contrast, it remained roughly the same in developing regions (Guttmacher Institute, 2016). Despite variations in abortion legislation, most women in Western countries are granted full or partial self-determination up until the 12th or the 20th week of pregnancy [21].

There is a complete ban on abortion (except for life-saving women) in Afghanistan, Angola, Bangladesh, Venezuela, Guatemala, Honduras, Egypt, Indonesia, Iraq, Iran, Ireland, Yemen, Colombia, Lebanon and, Libya, Mauritania, Mauritania, Mauritania , UAE, Oman, Paraguay, Papua New Guinea, El Salvador, Syria, Chile, Philippines.

In England, India, Iceland, Luxembourg, Finland, Japan, abortions are allowed only on medical and socio-economic grounds and in cases of rape.

«Pre-requisite» abortions in the early stages of pregnancy are allowed in the Union of Independent States and Baltic states, Australia, Austria, Albania, Belgium, Bulgaria, Hungary, Vietnam, Germany, Greece, Denmark, Italy, Cambodia, Canada, China, Cuba, Mongolia, the Netherlands, Norway, Romania, Singapore, Slovakia, Tunisia, Turkey, France, Czech Republic, Sweden, South Africa [22, p. 101].

On May 14, 2019, Republican-controlled Alabama's Senate of the United States passed the most violent abortion law in the US, which imposes a near-total ban on abortion. By law, abortion is a crime, even in the case of rape or incest (sexual intercourse between close blood relatives – parents and children, siblings). Doctors who perform this procedure are in danger of being imprisoned for a period of 10 to 99 years. An abortion can be legal only if the mother's life is at risk or the fetus is not viable. However, on October 29, 2019, a US District Court judge blocked this controversial law [23].

If we turn to the experience of Ukraine, on the contrary, there is no ban on abortions. However, the legislation contains numerous rules relating to medical abortion practices aimed at protecting the life and health of women. According to Part 6 of Art. 281 of the Civil Code of Ukraine, artificial termination of pregnancy, if it does not exceed twelve weeks, can be carried out at the request of a woman. In cases prescribed by law, artificial termination of pregnancy may be carried out at a pregnancy of twelve to twenty-two weeks. The list of circumstances permitting termination of pregnancy after twelve weeks of pregnancy is established by law [24]. In Ukraine, it is also possible to terminate the life (death) of one of the fetuses during multiple pregnancies through medical intervention (selective fetocide) in order to preserve the life of another fetus.

Of course, illegal abortion carries criminal responsibility. International law does not contain provisions on the prohibition or validity of abortions. In universal or regional human rights treaties, states, by virtue of numerous religious and ideological factors, try to avoid as much as possible any specific answer to the question of how long a human embryo can be considered a human. For example, according to the Convention on the Rights of the Child of 20 November 1989, a child means every human being below the age of eighteen years unless, under the law applicable to the child, the majority is attained earlier (Art. 1) [25]. The Convention for the Protection of Human Rights and Fundamental Freedoms and the practice of its application relates to establishing a balance between the interests of pregnant women and the legitimate need to protect the embryo to the powers of the States Parties to this Convention [11, p. 191].

It should be noted that in giving women the right to decide abortion independently, most countries did not take into account their husband's right to paternity (one of the reproductive human rights). International non-governmental organizations are of the opinion that free abortion is a violation of the child's right to life. This position is confirmed by the point of view of modern embryology.

If we consider the realization of the right to reproductive choice (the right to abortion) globally, it should also be noted that in some countries there is a tendency to develop criminal businesses in the collection and distribution of abortion material. It is stem cell hunting – embryonic or fetal – that pushes «businessmen woe» to campaign for abortion among the population. The Church view of abortion is unchanged – it is the sin of child molestation.

Considering the life of a person, which may end with the assistance of medical staff (assisted suicide, euthanasia), one should also mention such a phenomenon as a medical error. Indeed, the death of a person is possible from the health care itself. Medical error has been defined as an unintended act (either of omission or commission) or one that does not achieve its intended outcome, the failure of a planned action to be completed as intended (an error

of execution), the use of a wrong a plan to achieve a goal, or a deviation from the care process that may or may not cause harm to the patient. Patient harm from a medical error can occur at the individual or system level [26]. A medical error is legal, regardless of the consequences, with impunity. This is not a legal problem, but a medical one. Changing the concept of a medical error to a medical crime is unacceptable and leads to a destructive conflict of interest for patients and healthcare providers. Unlike in European countries, for example, statistics of medical errors are unfortunately not maintained in Ukraine. The nature of such errors in the world is different – due to the disorganization, poverty, unprofessionalism of physicians and the lack of general government management in this area.

In democratic countries, human life and health are recognized as the highest social value. That is why it is the direct responsibility of every state to ensure that effective jurisdictional mechanisms are in place to protect patients' rights, including those who have suffered from a medical error. The most effective jurisdictional mechanisms for protecting the patients' rights affected by a medical error are criminal law, civil law, and constitutional law mechanisms. At the same time, as international experience shows, there is also a need to create an effective system of non-jurisdictional mechanisms for the protection of patients' rights, which must include different insurance systems and alternative means of dispute settlement, in particular mediation [27, p. 2402].

The legal aspect of the human right to life requires the mention of such concepts as «necessary defense» and «extreme necessity» since everyone has the right to defend his or her life and health, life and health of others against unlawful encroachment. The necessary defense shall mean actions taken to defend the legally protected rights and interests of the defending person or another person, and also public interests and interests of the state, against a socially dangerous trespass, by inflicting such harm upon the trespasser as is necessary and sufficient in a given situation to immediately avert or stop the trespass, provided the limits of the necessary defense are not exceeded. Every person shall have the right to necessary defense notwithstanding any possibility to avoid a socially dangerous trespass or request assistance of other persons or authorities [28].

Infliction of harm to legally protected interests in circumstances of extreme necessity, that is to prevent an imminent danger to a person or legally protected rights of that person or other persons, and also public interests or interests of the state, shall not be a criminal offense, where the danger could not be prevented by other means and where the limits of extreme necessity were not exceeded [28].

Particular attention should be paid to the fact that international organizations have now begun to broaden the concept of the right to life, including not only the right to life as such. We can already see the weak so far, but the attempts to include in the understanding of this right the duty of the state to maintain and develop general conditions for a worthy life.

In view of this state of affairs and seeking to address the major problems related to the protection of the human rights to life, on 30 October 2018, the UN Human Rights Committee adopted General comment No. 36 on Article 6 of the International Covenant on Civil and Political Rights. Thus, the duty to protect life also implies that States parties should take appropriate measures to address the general conditions in society that may give rise to direct threats to life or prevent individuals from enjoying their right to life with dignity. These general conditions may include high levels of criminal and gun violence, pervasive traffic and industrial accidents, degradation of the environment, deprivation of land, territories and resources of indigenous peoples, the prevalence of life-threatening diseases, such as AIDS, tuberculosis or malaria, extensive substance abuse, widespread hunger and malnutrition and extreme poverty and homelessness. The measures called for addressing adequate conditions for protecting the right to life include, where necessary, measures designed to ensure access without delay by individuals to essential goods and services such as food, water, shelter, health-care, electricity and sanitation, and other measures designed to promote and facilitate adequate general conditions such as the bolstering of effective emergency health services, emergency response operations (including fire-fighters, ambulances and police forces) and social housing programs. States parties should also develop strategic plans for advancing the enjoyment of the right to life, which may comprise measures to fight the stigmatization associated with disabilities and diseases, including sexually transmitted diseases, which hamper access to medical care; detailed plans to promote education to non-violence; and campaigns for raising awareness of gender-based violence and harmful practices, and for improving access to medical examinations and treatments designed to reduce maternal and infant mortality. Furthermore, States parties should also develop, when necessary, contingency plans and disaster management plans designed to increase preparedness and address natural and man-made disasters, which may adversely affect enjoyment of the right to life, such as hurricanes, tsunamis, earthquakes, radio-active accidents and massive cyberattacks resulting in disruption of essential services [29, p. 6–7].

Everyone likes to live in a healthy environment which is a basic human necessity. A healthy environment is nature's gift. Air, water, and land are essential for all living beings. It has been recognized ever since Stockholm Declaration that both aspects of man's environment, the natural and man-made are essential to his well-being and to the enjoyment of basic human rights – even the right to life itself. The wholesome environment in the context of the right to life is a basic guarantee for the growth and development of individuals, society, and the nation itself. Human beings should be a central concern for sustainable development, and that they are entitled to a healthy and productive life in harmony with Nature [30, p. 79].

In order for a person to live a full life, he must be healthy. Healthy lifestyles are a lifestyle of every person aimed at preventing diseases and promoting health. Interesting is the Constitution of Hungary and Portugal. The Constitution

of Hungary defines the right to the physical and mental health of a person. Hungary has enacted the Constitution on access to healthy food and drinking water, environmental protection, and the provision of systematic physical education. Sanitary education is prescribed as the basis of basic knowledge of citizens about the care of their health. The Portuguese Constitution defines the steps that the state must take to ensure the right to health care. These are areas of financial support for health care, medical services and the production of medicines and medical products. It also mentions the responsibility to protect and strengthen the right to health. Consequently, it establishes not only the right but also the responsibility of individuals for health care [31, p. 1338–1339].

The text of the European Convention on Human Rights does not contain a separate article defining the human right to health, but let's consider how then the ECHR resolves the issue of violating the right to health care. Let's begin with access to experimental treatment or a remedy in the case of Hristosov and others v. Bulgaria [32]. Ten applicants who had cancer were complaining that they were denied access to unauthorized experimental cancer therapies. In accordance with the law of Bulgaria, such a permit can be issued only if the medicine has been authorized in another country. While medications were allowed for «philanthropic use» in some countries, they were officially not allowed. Accordingly, the authorities of Bulgaria refused to issue a permit [31, p. 1340].

The ECHR ruled that Article 8 (right to respect for private and family life) of the European Convention on Human Rights had not been violated. Given the limitation of the patient's right to respect for private life, as provided for in Article 8 of the Convention, the tendency was to provide the possibility, in exceptional circumstances, of the use of unauthorized medicine in European countries. However, the court acknowledged that this consensus was based not on the consistent principles of the legislation of these countries and did not extend to the precise order governing the use of such drugs. The Court also held that Article 2 (right to life) and article 3 (prohibition of torture and inhuman or degrading treatment) of the Convention were not infringed in this case [31, p. 1340].

Case J.N. and others v. Italy [33] concerned the infection of the applicants or their relatives with the AIDS or hepatitis C. The interested parties suffered from hereditary disorders (thalassemia) and were infected during blood transfusion, conducted by the State Health Service. The applicants complained, in particular, that the authorities did not carry out the necessary screening to prevent infection. They also complained about shortcomings in the subsequent civil proceedings and the refusal to pay them compensation. In addition, they claimed to have been discriminated against in other groups of infected individuals. The Court held that Article 2 (right to life) of the Convention had not been violated in relation to the protection of life of applicants and their relatives, taking into account, that it had not been established that at the time of the proceedings the Ministry of Health was aware or one should be aware of the risk of transmission of ACID or hepatitis through blood transfusions, and it was impossible to determine from what moment the Ministry knew or should have been aware of the risk. The Court also held that there had been a violation of Article 2 of the Convention in respect of civil proceedings, given that the Italian judiciary, when considering disputed complaints under Article 2, was not able to provide an appropriate and prompt response in accordance with the procedural obligations of the State in accordance with the ruling. It has been found that there has been a violation of Article 14 (prohibition of discrimination), in conjunction with Article 2 of the Convention, by establishing that applicants, patients with thalassemia or their heirs were discriminated against in comparison with hemophiliac patients who had the opportunity to use the extrajudicial decision of the case proposed by the Ministry [31, p. 1340–1341].

Thus, the human right to health and the human right to life are very interrelated. In the case of the consequences of failure or inadequate provision of medical care are more substantial, reference should be made to the violation of Article 2 of the Convention – if the life of the person ended.

The right to life – broadly understood as a right to be free from deadly violence, maiming, torture, and starvation. The right to life is paramount. It is much more narrowly crafted than the right to many entitlements that improve life (e.g., health, housing, and education) but are not required for us to remain alive [34].

In addition, oncology remains the most painful problem in the world. Cancer ranks first among diseases that take a person's life.

For example, an analysis of the current situation in Ukraine makes it plausible that the lack of an effective early cancer prevention system due to the underfunding of the health care system and the lack of effective management in this area could be considered a violation of patients' rights. The right to health is violated by restriction of the right to early diagnosis in order to detect cancer in the early stages. Moreover, such restriction in many cases leads to violation of the right to life, taking into account the specificity of disease progress and the possibility of its treatment [35, p. 1109].

Unfortunately, the foregoing suggests that individual states do not pay sufficient attention to the need of effective public health policy. In today's world, there are objective prerequisites for changing the system of protection of patients' rights and, consequently, for changing views on health protection in general, especially in the part of functioning of diagnostic procedures system. However, in order to have an effective regulative impact on the content, the nature and intensity of activities in the field of health care there must be some kind of preliminary practice of state authorities in: studying the state and dynamics of these public relations; their legal assessment; prognostication of consequences from neglecting of processes in the field of providing the right to health; elucidation of resource and instrumental possibilities of the state concerning effective influence on protection of citizens' rights to health and life. Meanwhile, it must be admitted that the formation of a state policy on ensuring the rights

of citizens to health and life, taking into account the various consequences of such a policy, cannot be narrowed down only to the proclamation of such rights, but also requires planning and development of relevant state programs. Analyzing the content of the declarative documents on the right to health and life, which is recognized as a typical form of state policy statement, it should be noted that their provisions should become a key source of law-making activity of the state in the relevant field of legislative regulation, and in the broader sense – the source of the organization of legal impact on health care relations. Failure by the state to provide the proper organization of health care through the establish ment of early diagnosis for cancer patients, considering wide incidence and mortal danger of cancer in case of late diagnosis, should be considered as a violation of human rights. The analysis of the practice of the ECHR makes it possible to state that such inaction of the state is an obvious violation of right to life (article 2), prohibition of tortures (article 3) and right to respect for private and family life (article 8) [35, p. 1112–1113].

Finally, it should be noted that we should agree with Dr. Mohd. Yousuf Bhat's and Dr. Syed Damsaz Ali Andrabi's opinion that the right to life is, therefore, the most fundamental of all rights, as it is the very core of humanity. It means a claim to so live that existence does not jeopardize the existence of others. It is not the only responsibility of individuals alone but State is a bigger partner in preserving the environment and in the realization of the right to life with human dignity. It is essential to create a shared international vision of long-term goals and to build the international frameworks that will help each country to play its part in meeting these common goals. There should be compatibility between the environment and economic development. Living standards beyond the basic minimum are sustainable only if consumption standards everywhere have regard for long term sustainability. Industrialized countries have an obligation to lead developing countries by shifting to sustainable development paths that would lead to a significant reduction in greenhouse gas emissions; promoting aggressive research on environmentally sustainable technologies; transferring such technologies to developing countries; and making large investments in climate-friendly technologies in developing countries. There should be a monitoring and reporting mechanism to provide a repository for information on compliance with universally accepted norms and a continuous and transparent effort [30, p. 84].

CONCLUSIONS

The right to life is a fundamental, inalienable human right that belongs to civil (personal) rights in the system of human and citizen rights and freedoms. This right consists of the following elements: the inalienability of the human right to life, the prohibition of arbitrary deprivation of life, and the right to protect one's life and that of others against unlawful encroachment. A person has the opportunity to manage his life at his own discretion, first of all, to use all legitimate means and resources for the prevention of premature mortality, etc. The duty to protect the right to life, in particular, requires that states take appropriate measures to remedy the general conditions in society that may endanger lives or prevent persons from living a dignified life.

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REVIEW ARTICLE

FEATURES OF THE ORGANIZATION AND PROVISION OF EMERGENCY MEDICAL CARE IN POLTAVA REGION

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ABSTRACT

The aim: Is to study and analyze the dynamics of the indicators of the emergency medical service of the Ukrainian and the Poltava region in the context of the reforms of the healthcare system in Ukraine.

Materials and methods: In this work, the indicators of development of the emergency medical service of the Ukrainian and the Poltava region were studied and analyzed.

Conclusions: The provision of emergency medical care in the Poltava region is provided by the Poltava Regional Center for Emergency Medicine and Disaster Medicine. The structure of the center includes 4 emergency medical stations, which are located in cities such as Poltava, Kremenchuk, Lubny, Mirgorod. A modern telemedicine center was built in 2018 for emergency counseling on-line in new directions was carried out: ultrasound and endoscopic diagnostics, radiology, counseling during surgical interventions, laboratory diagnostics, etc. Emergency medical care reform Poltava Regional should be aimed at increasing the efficiency of the use of resources; provision of the EMC system by the relevant vehicles; qualitative training of doctors in emergency medicine; informatization of the EMC system.

KEY WORDS: emergency medical care, health care system, reformation of health care system

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INTRODUCTION

Social and economic problems in Ukraine had a negative impact on the health care system and caused a difficult demographic situation in the country. Year after year, a lot of people die due to the lack of timely qualified medical care, this is especially true for patients with acute myocardial infarction [1, 2].

The main reasons of this situation are: improper organization of the network of emergency medical care centers and patient routes, technical condition of ambulances and low financial support. This showed the need for quick measures to reform the medical industry and, especially, the emergency medical service. The government pays particular attention to the improvement and development of this part of the medical sector [3, 4]. It is known that the life expectancy of patients depends on the work of ambulance, the active working period of people extends and their quality of life improves [5].

THE AIM

To analyze the state of the emergency medical care condition of Poltava region during the reform of the emergency medical care in Ukraine

MATERIALS AND METHODS

The analysis and systematization of indicators of the condition of emergency medical care in the Poltava region. Medical and statistical methods were used to collect, process and analyze data.

REVIEW

Analyzing the state of resource provision of the emergency medical care system in Ukraine, it should be noted that today, emergency medical care is provided by 2954 ambulance teams, of which 1867 are paramedic teams. It should be noted that emergency medical aid teams are staffed with doctors by 62%, and junior specialists with medical education by 86% [6].

The structure of ambulance brigades and the time of arrival of the brigades to the call are shown in Table I.

During the study period, the number of emergency medical aid teams practically did not changed, but there was an increase in paramedic teams with a reduction in general medical and specialized medical teams. The indicator of the timely arrival of the ambulance team on call in the city and countryside has decreased, possibly due to the need to update the park of specialized ambulances.

The provision of emergency medical care in the Poltava region is provided by the Poltava Regional Center of Emergency Medicine and Disaster Medicine.

The center provided emergency medical assistance to the population of the region of 1,405,99 thousand people, including 867,200 urban population and 538,800 rural people.

Table I.. The dynamics of the number of emergency medical teams and staffing of emergency medical care transport in 2017-2019.

Year	Emergency medical teams (absolute number)				The arrival time of the emergency medical brigade, %	
	Total	Specialized ones	General medical care professionals	Medical paramedics	Done	Up to 20 min
2017	2922	137	994	1790	93,3	87,8
2018	2948	138	989	1824	91,6	88,8
2019	2955	109	1000	1845	92.3	85,4

The structure of the center includes 4 emergency medical stations, which are located in such cities as Poltava, Kremenchuk, Lubny, Mirgorod, which include 24 substations and 22 points of permanent and temporary deployment of teams. The center for emergency medical care and disaster medicine is staffed by 75% [7, 8, 9].

The staffing rate of the Emergency Medical Center with individuals is 69% (in 2017 -70%), and staff positions are occupied by 75%. It should be noted that the percentage of medical workers with a qualification category is 77%. At the same time, in 2018, 67 doctors and 214 junior specialists with medical education took advanced training courses. The educational department trained 162 medical specialists (including Kremenchug, Lubensky and Mirgorodsky districts).

Ambulance crews are equipped with new medical equipment. It was received from the World Bank's funds under the project "Improving Health Care in the Service of People" (subproject "Implementing an Innovative Model for the provision of services for patients with hypertension in Poltava Region"). Were received two modern ambulances and 100 electrocardiographs with remote data transfer, which made it possible to increase the detection rate of acute cardiac pathology [2, 3].

According to the results of the work of the regional emergency medical service in 2018, the teams of the Poltava Regional Center for Emergency Medicine and Disaster Medicine served more than 314,000 emergency requests a medical doctor, what is 10.4% less than in 2017 (more than 350,000 calls).

That was achieved by reducing the number of unreasonable calls by providing the public with information about the work of the emergency medical service. Generally, the share of calls to the countryside increased – 26,7%, instead of 26,3% during the past year, which had a positive impact on the provision of medical care to the rural population.

During the visit to the site of the brigades of the Poltava Regional Center for Emergency Medicine and Disaster Medicine, more than 300,000 people were provided with medical assistance in 2018. At the same time, about 26,000 patients were provided with outpatient care at the Emergency Medicine Center in 2018.

In 2018, in the Poltava region, the number of people assisted by the emergency medical service increased by 62,2% (186.974) people, compared with 60.5% in 2017. In 2018, the share of people delivered by ambulance teams for hospitalization increased to 26.8% (84,339 people) of the total number of visits compared to 25.3% (88,900 people) in 2017, which improved the quality of medical help. In 2018, 5.1% (4,319) people were delivered to medical directions of medical institutions, which is more than in 2017 – 3.9% (3,499) people.

The number of visits by ambulance crews that ascertained death was 3.063 in 2018, which is 1% of the total number of visits and, at the 2017 level, 1% of the total number of visits (3,291 visits).

If we consider the indicators of the timely arrival of teams to the patient, then there have been no significant changes, so in the city, 97% of emergency medical care teams arrived at the patient within 10 minutes from the moment of connection in 2018 (2017 – 96.8%). In rural areas, 96.8% of emergency medical teams arrived to the patient within up to 20 minutes from the moment of connection in 2018 (2017 – 96.4%). As modern studies show, the timely arrival of the emergency medical team to the patient significantly reduces the risk of complications of the disease and the percentage of mortality, including in acute cardiovascular pathology [10].

In 2018, 1,085 advisory visits to medical institutions of the region were completed and 376 patients were delivered to institutions of the third and fourth levels of medical care.

Electronic registers of applications to medical institutions of the Poltava region for medical assistance from displaced persons from certain areas of the Luhansk and Donetsk regions are maintained. In 2018, more than 15 thousand such requests were registered. It also maintains registers of emergencies that have occurred in the region, and the number of volunteers trained in medical care skills.

In accordance with the Law of Ukraine "On Emergency Medical Aid" and the national project "Timely Help", the operational dispatching service of the emergency medical center functions. In 2018, the phased connection of 10 substations of the largest district of the center to the call routing service to the short number "103" using the modern SIP-trunk protocol was completed. In these areas, the introduction of a remote workstation for the head of the visiting team of emergency medical care with work on tablets with a mobile application was ensured.

A modern telemedicine center was built in 2018 for emergency counseling on-line in new directions was carried out: ultrasound and endoscopic diagnostics, radiology, counseling during surgical interventions, laboratory diagnostics, etc. Taken the measures to implement the work of a portable ultrasound machine on emergency health care teams for emergency diagnosis at the prehospital stage.

DISCUSSION

Thus, we see that the staffing of emergency medical aid teams with doctors is insufficient [6]. There is a personnel problem in the staffing of the Poltava Regional Center for Emergency Medicine and Disaster Medicine, and insufficient qualification training of medical personnel.

Funding and provision of the center with equipment and vehicles is inadequate and is partially compensated by the assistance of the World Bank in the framework of the project "Improving Health in the Service of People".

Despite this, there have been positive changes in the system of emergency medical care in the Poltava region, especially the rural population. Thus, the number of unmotivated calls to emergency medical teams decreased, the number of episodes of emergency medical care, including those with acute cardiovascular pathology, increased, the number of specialized care increased, and the time of arrival of the emergency medical team decreased.

Also, the connection of substations to the call routing service using the modern SIP-trunk protocol and the introduction of a remote workplace for the head of the ambulance team were completed. The telemedicine center allows them to provide emergency medical care on-line.

As we can see, the Government of Ukraine is actively continuing to reform the medical industry in the country. An important area of medical care is improving the quality of emergency medical care in Ukraine [4, 5].

The main problems of the ambulance service that require improvement are the legal framework, informatization and use of the electronic document management system, the interaction of all structural units of the emergency medical care center, the correct work of personnel and the availability of modern vehicles [4, 5]. The development of informatization of the health care system and especially the ambulance sector is one of the important areas of reform at the present stage.

CONCLUSIONS

Thus, reforming the emergency medical care of the city of Poltava will significantly improve the quality and availability of emergency medical care urban and rural population. In the future, emergency care reform should be aimed at: improving the efficiency of use of emergency health care resources; increasing the availability and quality of emergency health care; the population and health workers should be positive about emergency health care reform; providing the emergency health care system with modern vehicles; ensuring the quality training of emergency medical doctors to the extent necessary for serving the population; improvement of the system of professional development of junior medical specialists of the emergency health care centers; development of new methods of employee motivation.

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REVIEW ARTICLE

OVERVIEW OF WELLNESS METHODS FOR PEOPLE PRACTICING SPORTS

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ABSTRACT

The term "wellness" embraces a wide spectrum of methods that impact the human body by restoring its capabilities and functions, which were previously depleted as a result of increased physical and mental activities such, i.e. sport. Judging by the number of amateur, semi-professional, and professional sporting events at local and national levels, societies consider sports a major part of their everyday lives. A growing percentage of the population is exposing their bodies to various strains, which may result in fatigue, overtraining and injuries, and so the market demand for recovery-related services is on the rise. Therefore, this paper is an overview of the most important and the latest wellness systems and methods applied in today's sport. They divide into three areas: pedagogical, psychological and medical-biological. Among the most popular treatments are: light radiation with infrared rays, cryotherapy, electrotherapy, magnetotherapy, ultrasound and laser therapy, as well as a sauna, paraffin compress, mud compress and brine baths. In a broader context, the paper also acknowledges the growing demand for better body recovery methods and the latest developments in the field of sport physiotherapy.

KEY WORDS: wellness, recovery, sport, physical therapy

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INTRODUCTION

The term 'biological regeneration' is often understood in a rather general way and often used interchangeably with e.g. the word 'SPA' or 'wellness'. Contrary to its common understanding, specialist literature specifies the term as follows:

This term is understood as a number of effects (...) on the human body aimed at counteracting overstrain and restoring full physical fitness and mental motivation which have been deteriorated due to exhausting physical and mental work, local fatigue of mainly the musculoskeletal system, convalescence, etc. (Giermek, Dec 2007)

In a slightly broader sense, biological regeneration is applied in three areas:

A group of psychological, educational and biological effects, the aim of which is to activate the relaxation process, which is closely related to the protection and enhancement of health; (Kasprzerczyk, Fenczyn, 1996).

THE AIM

This paper presents an overview of established and novice therapies of biological regeneration in the context of growing popularity of various forms of physical exercise.

REVIEW AND DISCUSSION

HISTORY AND THE PRESENT

Interestingly, restoring the body to its healthy condition and functionality from previous intensive physical activity is not a modern invention. It originates in antiquity, where sport played an important role in life and culture of, for example, the Greeks. High physical fitness was perceived by the Greeks as a proof of human perfection and a way to live a vital, healthy life. It is certain that already in those days, wellness treatments were used to improve the body's efficiency and recovery, because apart from artists or philosophers, the players were also accompanied by physicians. Hippocrates himself watched training sessions to better understand the human body and to develop better dietary recommendations. Dissertations were written on training regimes, and Galen (2nd/3rd century AD) came to the conclusion that medics must train equally hard as athletes with whom they worked, in order to achieve excellence in their medical field [1].

THE GROWING ROLE OF BIOLOGICAL REGENERATION

Today, sport is not only a form of spending free time, relaxation, detachment from everyday life. Society is becoming increasingly aware and increasingly often experiences its beneficial effects on mental and physical health. It can already be noticed that both young and elderly people as well as entire families are often present at various events, health paths where they try to push the limits of their endurance. This in turn means that the percentage of people experiencing fatigue, overtraining and injuries will inevitably increase. As a consequence, there will be a growing demand on the market for effective regenerative treatments, and the

effectiveness of such treatments rely on expert knowledge about applying biological regeneration therapies and specialized equipment. It can be safely assumed that the growing number of amateurs and semi-professionals will be aware that biological regeneration applied at various stages of training – not only in the case of fatigue or more serious injuries – prepares the body to achieve better physiological results. All of the above aspects mean that in the field of biological regeneration there is a growing demand for a) specialists who know how to apply such methods in the context of sport and b) for increasingly effective methods of body regeneration. Therefore, it is worth reviewing the most important systems and methods of biological regeneration as well as looking at the directions of development of this increasingly important field of medicine.

SYSTEMS OF BIOLOGICAL REGENERATION

Biological regeneration encompasses tools that can be divided into three areas: educational, psychological and medical-biological [2, 3].

1. Educational measures:
- creating training plans that are optimal in terms of the athlete's abilities
- proper planning of training cycles
- accounting for balance between exercise and rest in training
- including specialist training sessions aimed at providing the best conditions for physical and mental relaxation
- achieving motivation levels sufficient to mobilize and activate the mind and the body [3].

2. Psychological measures:
- developing conditions conducive to building good interpersonal relations, e.g. between a player and the team, coaches, doctors, etc.
- providing special forms of rest and relaxation which eliminate players' mental strain
- strengthening mental resilience with appropriate cooperation with the coach and psychologist [3].

3. Medical and biological measures:
- adapting dietary plans to the current needs of the athlete
- ensuring proper healthcare
- applying physical medicine treatments and spa therapies [3].

AIMS OF WELLNESS

Skilfully applied biological regeneration can increase the effectiveness of each training stage. In this regard, its tasks can be divided into essentially four elements:

1. Planning out post-exercise recovery at the stage of creating a training plan, which is aimed at more efficiently remedy functional disorders [3, 4].
2. Recovery optimization: selection of appropriate therapies which accelerate the regenerative activities of an athlete's body to restore them to their current needs
3. Prevention: proper training and training methodology and physical activity as well as eliminating the effects of effort, among others, the production of defence and adaptation mechanisms.
4. Completing healing processes of suffered injuries that often lead to long absence from sports activity. The most common are bruises, skin abrasions, and damage to the capsulo-ligament apparatus [3, 4].

Biological regeneration, due to its wide application possibilities, is used and should be used in every training phase and selected according to the type of physical activity. The most common treatments of biological regeneration include: infrared irradiation, hydrotherapy, cryotherapy, heat therapy, electrotherapy, magnetic therapy, ultrasound therapy, massages and balneotherapy.

HYDROTHERAPY

It is probably the most often used type of physical therapies applied in post-exercise recovery process. Water-based treatments are in essence mechanical stimulation of the body through various levels of pressure and a broad range of temperatures as well as a beneficial effect of water resistance and buoyancy. The most important effects of hydrotherapy are local and systemic reactions of the body to heating, cooling, local mechanical stimulation (e.g. showers, hydromassage) and reaction to immersion in water due to water's relieving and resisting effects. Overall, the goals of the various hydrotherapy approaches can be summarized as striving to reduce chronic pain and inflammation, muscle tension disorders as well as improving coordination and mobility [5-9].

CRYOTHERAPY

The beneficial effect of systemic cryotherapy comes from reactions of a body subjected to very low temperatures of -100°C to even -160°C for about 2-3 minutes. Systemic treatments take place in specially designed rooms, e.g. cryo-chambers or cryo-saunas where the room is cooled with liquid nitrogen. Local treatments, on the other hand, use e.g. ice, cold water, frozen gels, compresses or airflow of liquefied CO_2. The goal is to cause physiological stress, in which first the peripheral blood vessels and muscles contract and the blood flow and metabolism slow down. Then the body in a defensive reaction rapidly dilates the vessels increasing blood flow. As a result, the amount of nutrients, oxygen and anti-inflammatory mediators supplied to the cells increases. These reactions make cryotherapy an excellent solution for relieving pain, swelling and inflammation states, stimulating regeneration of damaged tissues, relaxing muscles and increasing the range of motion. It has a positive effect on metabolism and stimulates the nervous and immune systems. It often clearly improves also the well-being of active people who underwent surgical operation [5, 6, 8-13].

Indications for cryotherapy are e.g.:
- rheumatological and degenerative diseases,
- post-traumatic conditions,

- lesions resulting from overstraining the musculoskeletal system,
- in biological regeneration, e.g. in the case of athletes, with physically and mentally fatigued people and in myofascial pain syndromes [5, 6, 8, 14].

HEAT-BASED THERAPIES

The sources of heat in biological regeneration can be warm water or heated dry or humid air. Therapeutic treatments can be used locally or systemically. The body's primary reaction to the heat is a the reaction of the blood vessels. As a result of the heat, the blood vessels of the skin and those that supply blood to the kidneys, spleen and brain expand and, in accordance with the Dastre-Morat law, large vessels of the chest and abdomen react antagonistically and constrict. An opposite pattern occurs when applying cold therapies. Constricting vessels inside the body lead to reduced blood circulation and reduced oxygen supply to deeper tissues, which triggers the desired adaptation – the number of red blood cells increases, the overall oxygen management improves and the number of capillaries in internal organs and muscles increases. During systemic treatments (e.g. bathhouse, sauna), the body gets overheated, which entails a number of effects: profuse sweating affects the water and mineral balance of the body, metabolism increases (body temperature increase by 1°C = rise in metabolism by approx. 3.6%, which for example in the case of sauna means a rise by 11%), heartbeat and breathing pace accelerate. In the context of sport, one of the most beneficial effects of overheating is reduced muscle tension. It has also been proven that, e.g. during sauna treatments and up to a dozen or so hours after treatment, the pituitary and adrenal glands secrete hormones. The adrenal glands increase cortisol production, which, among others, increases exercise capacity [5-7, 9, 11]. For local treatments, in order to achieve a reaction some of the equipment used are e.g. electric cushions, thermophores or heating lamps (e.g. sollux). Blood and lymphatic vessels expand when exposed to heat. Such increased blood flow has beneficial effect e.g. in inflammation treatment. Heat relieves pain and reduces muscle tone of skeletal and smooth muscles. Therapies using sollux lamps are used in chronic and subacute inflammation of joints and periarticular tissues, post-injury pathologies of the musculoskeletal system, myalgia, and in tendon, ligament and muscle disorders due to biomechanical overload. It is also recommended to use infrared radiation to warm the skin and any cold areas of the patient's body before massage, kinesitherapy and stimulation with electricity [6, 7].

ULTRASOUNDS

Ultrasounds (sound therapy also known as sonotherapy, phonotherapy, ultrasound therapy, ultrafonotherapy) are acoustic waves inaudible to the human ear, i.e. above 20 kHz, and therapies use a range of approx. 800-2400 kHz. Ultrasound therapy is one of the most widespread therapies all around the world, just as in Poland. Interestingly, it is also one of the best-studied methods of physical therapy, both in terms of the physical phenomenon of ultrasound and its effects and effectiveness. Unlike the methods of physical therapy described so far, exposure to sounds is primarily executed using local procedures (primary ones), which indirectly affect other parts or the entire system, and in such case their effect is described as secondary. Local action on tissues is a complex process which consists of three main aspects:

a. Physicochemical effect: physicochemical changes induced by ultrasound depend mainly on the intensity of the wave, and then on other parameters such as the duration of the procedure or the type of tissue. Significant effects include acceleration of protein breakdown, transformation from gel to sol, and increased electrical conductivity. Most of the chemical reactions that result from sound treatment are oxidation.
b. Mechanical action: When the acoustic wave propagates, it causes the particles of the medium (e.g. air, water) to vibrate, therefore an acoustic wave is also a mechanical wave, therefore the way sound waves affect gases, liquids and solids is mechanical, physical. In this context, the effect of ultrasound on tissues is referred to as micro-massage, and its effect on cells is e.g. a change in cell volume by 0.2%, which is a significant change.

Summarizing the two points above, the athermal effect of ultrasound stimulates the flow of intra- and extracellular fluids as a result of changing pressure and increased permeability of cell membranes. As a result, there is an anti-inflammatory effect and repair processes accelerate.

c. Thermal action: One of the effects of mechanical action is generation of thermal energy, which depends on the parameters of ultrasound used, exposure time and the level of wave absorption by a given tissue. The thermal effect is strongest in the border area of heterogeneous tissue structures, e.g. bone and muscle tissue. Next, the most susceptible to the overheating effect are the nervous tissue, slightly less muscles, and the least susceptible is the adipose tissue. In practice, this means that the therapy is suitable for tissues with high collagen content, such as tendons, ligaments, joint capsules – heating increases their elasticity without reducing their strength [6, 10, 11, 15, 16].

SHOCKWAVE THERAPY

One of the varieties of ultrasound therapy is the so-called Shockwave therapy. This is a relatively new method that was first used in the treatment of kidney stones, where it was found that in addition to crushing the stones, a side effect is relieving ailments related to the musculoskeletal system. The method is highly effective; it sometimes postpones and even prevents operations after injuries or degenerations of the locomotor system [10, 17, 18]. Due to its very fast and measurable effects, the method has a special place in sport and is primarily used for treating pain and inflammation,

and one of its first applications in biological regeneration during major sporting events was the Olympic Games in Atlanta in 1996 and at the football World Cup in France two years later. The procedure is a series of rapid increases in pressure: from 1500 to 3000 wave shocks up to 21 times per second. The medium of propagation of such waves is most often water, and to facilitate transmission deep into the tissues are used for example so-called ultrasound gels are used, for example. It is a very precise method as it gets applied onto a selected area and affects only or mainly pathological tissues. The impact of the wave is achieved even up to 12 centimetres deep into the tissue. The most common conditions treated with the shockwave are damage to the tendons and joint capsules, tendon attachment, patella pain, post-traumatic pain syndromes, myalgia, achilles injuries, shoulder and wrist injuries, patellar tendonitis and many others [10, 15, 17-19].

MAGNETIC THERAPY

The therapeutic factor in magnetic therapy is variable magnetic field for which a specific frequency and magnetic induction, i.e. field strength, are selected. One of its applications is treating muscle and tendon injuries and post-exercise muscle fatigue, because magnetic field penetrates all body structures, improves oxygenation and blood supply. In addition, the osteogenic effect accelerates broken bone healing process. Magnetic therapy is a non-thermal method of physical therapy, and due to the fact that magnetic field is able to penetrate most substances, it is thus effective also when the subject wears a cast. The shape of generated magnetic field pulses varies for different applications of the method, e.g. triangular pulses are used for regenerating articular cartilage, tendons and ligaments, and sinusoidal pulses are used for nerves and muscles. Frequency (between 1 and approx. 80 Hz) and the strength (between 0.5 and 10 mT) of the magnetic field are set up according to the condition, and lower values are applied for acute conditions and the highest for chronic conditions [20, 21].

MAGNETIC STIMULATION THERAPY

It is a form of therapy similar to magnetic therapy, but more modern and based on patented solutions. Magnetic stimulation therapy, unlike the magnetic therapy, uses a much lower induction (strength) and higher field frequency, which reaches even 3000 Hz (compared to a maximum of 100 Hz in magnetic therapy). The second significant difference is the shape of the magnetic field signal, which is described as sawtooth in the case of magnetic stimulation, while in magnetic therapy it takes different geometrical shapes [6, 7, 20, 21]. Magnetic stimulation relaxes smooth muscles in the walls of blood vessels, has an analgesic effect, accelerates regenerative processes, wound healing, stimulates bone formation, increases bone mineralization while inhibiting demineralisation processes and has an anti-inflammatory effect when stimulating the production of E and c-AMP prostaglandin. It is an increasingly popular form of biological regeneration, which may be even applied at home due to the increasingly popular magnetic devices (Viofor JPS) as they show high rates of safety in application [20-25]

VIOFOR JPS SYSTEM

Therapy with the Viofor JPS system – one of the certified (CE) devices in magnetic stimulation therapy – consists in affecting the body with a slowly changing magnetic field, which leads to changes in the body that support biological regeneration processes. As a consequence, mechanisms are launched in the body which clearly facilitate restoring homeostasis. This therapy uses a pulsed magnetic field of very low frequencies. The Viofor system, thanks to its appropriate dimensions, can be used in all conditions: at home, while travelling, before and after doing sports. Treating with magnetic stimulation can be carried out at any stage of the training cycle, even during breaks, and the best results are achieved by using magnetic stimulation after training (e.g. oxygen debt decreases, blood saturation improves, and muscle tone decreases). It is recommended to use the Viofor system in the following cases:

- pain,
- swelling,
- injuries of the soft tissue,
- injuries of joints and hard tissues,
- inflammatory conditions,
- neurosis, mood disorders,
- preventive sedative effect,

aimed at counteracting stress
of everyday life,

- stress, especially after prolonged

mental tension (which is part of competing in sports),

- attention deficit disorders
- improves oxygenation of the body – better

utilization of oxygen [20-25].

ELECTROTHERAPY

The aim of electrotherapy is to reduce pain and improve blood supply to stimulated tissues. It accelerates resorption of intra-articular oedema and effusions [11]. These treatments are performed with the use of direct or alternating current of varying frequency. Treatments based on the use of direct current are **galvanization** and **ionithermie**. In galvanization, depending on different electrode positioning, different therapeutic effects may be achieved which cause muscle relaxation (anodic electrode) or their stimulation (cathodic electrode) [6, 10, 11, 16]. The flow of direct current through the tissues is accompanied by electrochemical, electrokinetic and electrothermal phenomena that increase the blood supply to the body stimulated with electricity as well as the threshold of nerve excitability and reduce their conductivity. Galvanic current increases absorption of hematomas, effusions and oedema due to increased vascular permeability, which is a result of secreting histamine. It improves blood circulation by having

a beneficial nutritional effect on tissues, has anti-inflammatory and analgesic properties (the 'control gate' mechanism), and also accelerates regenerative processes, which allows faster wound healing [11]. Galvanization is used, among others, in pain syndromes associated with neuritis, flaccid paralysis, bone union disorders, post-traumatic conditions, muscle overstrain and abnormalities in the functioning of peripheral circulation [6]. Another treatment that uses direct current is **iontophoresis**, in which drugs dissociating into ions are introduced directly into the tissues. Characteristic for this treatment is that the healing factor are ions introduced into the body, and not the current itself. That is why it is so important to choose the right drug in a given disease entity [5, 6, 11, 26]. The main goals to achieve in iontophoresis treatments are: analgesic and anti-inflammatory effects, improve blood flow, stimulate fluid resorption, change tissue structure, prevent the formation of adhesions and contractures, and stimulate tissue regeneration [27]. The next group are treatments using **alternating currents**.

Diadynamic currents arise as a result of rectifying sinusoidal alternating current with a frequency of 50 and 100 Hz. The pulse duration is 10 ms and is equal to the pause time [6, 10]. Diadynamic currents use sinusoidal pulses superimposed on direct current, in various combinations, depending on the required action, due to which they have analgesic and vasomotor properties, and also increase the excitability of the neuromuscular system. Beneficial results of diadynamic currents are used in such cases such as fatigue inflammations, tendon and muscle tears, in contusions, scar fractures and uncomplicated sprains. Whereas in combination with massage, balneotherapy and kinesiotherapy, they are applied to conditions after sprains and subluxations, in muscle atrophy and ankylosis resulting from inactivity of the joint, e.g. after long immobilization with a plaster cast [3, 6, 11, 19]. **The Träbert current**, also called stimulus massage, is a unipolar, pulse, rectangular, low-frequency (143 Hz) current. The pulse duration is 2 ms, and the break time is 5 ms. These currents reduce the activity of the sympathetic nervous system, which causes muscle relaxation. Their use is recommended, among others, in pain syndromes, increased muscle tension, atrophies, in post-traumatic conditions, in Sudeck's syndrome and hyperalgesia [28-30]. **Interferential currents** are medium frequency currents (1,000-10,000 Hz), and their advantage is that they affect the inside of treated tissues and not the tissues directly under the electrodes. These currents are often used in cases of people who actively play sports, most often 90-100 Hz frequencies are applied due to their analgesic properties and relieving stress in the sympathetic nervous system. These currents are made as a result of overlapping two sinusoidal currents [28, 30].

MASSAGE

The most common physiotherapeutic regeneration treatment among athletes is **a classic dry massage**. The massage uses all known techniques such as stroking, kneading or rubbing [3]. The aim of sports massage is to prepare for extreme physical activity as fast as possible, achieve maximum results and remove considerable body fatigue after exercise. Massage has a very strong effect on the nervous system, especially on its neuromuscular part, improves tissue trophism, affects the circulatory system, relaxes the body and mind, improves sleep quality, removes muscle and joint pain and accelerates the excretion of metabolic products (body waste). In competitive sports, various types of massage are used such as training massage, massage applied before the competition, pre-workout massage, post-workout massage and support massage. Thus, the classic sports massage has a warming, regenerating and healing functions. In biological regeneration, water massage (underwater and whirlpool), isometric massage and pressure massage are also used [3, 7]. **Underwater massage** should be used immediately after participating in sports competitions or hard training in water in the temperature range of 32-33˚ C, and treatment time should be 10 minutes. **Whirlpool massage** – it is a vibration massage, it utilizes mechanical and thermal stimuli in a water environment, which lead to muscle relaxation, reduce pain and improve blood circulation. **Pressure massage** is performed using specialized devices such as special legs, belts and cuffs. Limbs are placed inside them, which are alternately affected by elevated and lowered atmospheric pressure. The biggest advantages of this method are very good stimulation of blood circulation and increase of muscle strength, and the fact that metabolic products are removed from the muscles. Stimulated blood circulation gives the desired feeling of lightness in the limbs subjected to massage, which is especially appreciated by runners. Pressure massage treatments are a great complement to a warm-up or end of training. Due to its high efficiency in improving strength and increasing muscle mass, pressure massage is an important supplement to training [3, 6-8]

INDIBA® ACTIVE IN SPORTS MEDICINE

It is one of the state-of-the-art methods currently in use in physical therapy. It has been developed by Spanish specialists, and its effectiveness in sports, rehabilitation and aesthetic medicine has been thoroughly documented. The core of the method is a patented device which consists of two special electrodes that deliver thermal and non-thermal current to tissues and cells during short, maximum 15-minute sessions. This method uses the high frequency current of 448 kHz, which triggers a number of changes in tissues (it changes the permeability of the cell membrane, increases metabolism and oxygen demand). A big advantage of this therapy is the fact that when combined with manual therapy it can affect soft, cartilage and bone tissues. The main goals of the therapy are faster tissue reconstruction, lowering the pain threshold and reducing inflammation. The Indiba® Activ method causes reactions in the body at the cellular level and forces it to create new, healthy cells, thanks to which tissue regeneration takes place faster. This results in reduced pain, it significantly reduces inflammation, and thus reduces the duration of treatment. Even tissues affected by chronic inflammation

(a cause of many chronic conditions) are stimulated. Affecting the three types of tissues, referred to above, occurs by using two systems in two separate electrodes, which is the basis of the device's operation:

- resistive electrode – affects bone tissue, tendons and ligaments
- condensation electrode – affects soft tissues, mainly muscles, and connective tissue

In light of the above benefits, the Indiba® Activ method is successfully used in sports due to:

- reduction of pain
- reduced susceptibility to injuries
- faster recovery after exercise
- better physical preparation [31].

The method is used by Europe's top football clubs such as FC Barcelona, Liverpool FC, and also there is research collaboration with prestigious medical centres such as: Hospital Ramón y Cajal in Madrid, CIMA and QUIRON Hospitals in Barcelona, Santa Cruz and Sant Pau Hospitals in Barcelona, University Clinic in Valencia, CONI Medicine, Research and Sport Institute in Rome and Gaetano Pini Institute in Milan.

BALNEOTHERAPY

Balneology is a field of medicine that uses natural healing resources such as mineral water, medicinal gases, peloids and favourable climatic conditions. Currently, it is noticeable that balneotherapy is applied increasingly often in sports medicine and biological regeneration, especially in the prevention and treatment of injuries of athletes from various disciplines. Spa treatment increases the intensity of adaptive mechanisms, activates the body's functional reserves, their action is directed at the entire system, and therapeutic results after application last longer and are more permanent. Balneotherapy treatments are based on the properties of: medicinal materials, weather conditions, optimal living and relaxation conditions, and physical therapy equipment, which is an important addition to athletes' recovery processes [6-8]**.** In countries where sports culture is significantly developed and a large number of medals during sports competitions (World Championships, Olympics) are won, such as e.g. USA or Germany, there are a lot of rehabilitation centres for sports people, which were established many years ago. Currently, the same is true for Poland, where facilities dedicated to athletes are built with a wide range of balneo-treatments for biological regeneration, and which are gaining recognition and popularity, e.g. COLUMNA MEDICA in Łask near Łódź.

CONCLUSIONS

The popularity of sport is undeniably growing and is expressed in the increasingly accessible infrastructure, numerous blogs and social media channels devoted to training, or even increasingly dense network of gyms, clubs and mass sports events. Together with the increasing number of people who train, the demand for effective biological regeneration naturally rises, hence this article provides an overview of the most important therapies in the context of regenerating the body after physical exercise. Bodies of people actively practicing sports experience various levels of strain, fatigue, overtraining, sometimes injuries, and the specificity of such conditions varies in every sports discipline. In addition, biological regeneration methods are used as a prophylaxis and a way to increase the body's capabilities – it is more than just post-workout recovery, resting and relaxation. All this makes it necessary to know a wide range of therapeutic treatments and, equally important, to have an in-depth knowledge about individual therapies, because each procedure may be applied with various intensities and exposure times. In addition, one of the factors determining the selection of the right treatment at a given moment will be the need for mental recovery of the athlete. What is significant is that the variety of methods available allows avoiding falling into routine in conducting wellness.

Certainly, further research is needed to better understand the processes occurring in a body that undergoes physical therapy, to improve the effectiveness of existing methods, and to seek new therapies.

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Conflict of interest

The Authors declare no conflict of intererest.

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REVIEW ARTICLE

NIKOLAI IVANOVICH PIROGOV – AMICUS HUMANI GENERIS

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ABSTRACT

The aim: Was to analyze the scientific and pedagogical heritage of N.I. Pirogov through the prism of his outstanding polymathic abilities.

Materials and methods: The authors examined the scientific and pedagogical heritage of N.I. Pirogov using the method of historiographical analysis, as well as the methods of synthesis and generalization. The study relies on research publications devoted to N.I. Pirogov's biography, as well as his epistolary and autobiographical works.

Conclusions: The scientific novelty of the research is that the biography of N. Pirogov is represented with refinements and additions based on his latest work "From the Diary of an Old Doctor". The authors analyzed the epistolary heritage of N. Pirogov, which served as a valuable biographical source. Given the anthropocentrism of the current stage of the existence of society and European civilization, the authors sought to "revive" the biography of N. Pirogov. In this regard, considerable attention is paid to his personal life. As a result, the article considers this outstanding personality in a new perspective, presenting the main stages of his scientific and medical activity.

KEY WORDS: N.I. Pirogov, higher education, scientific heritage, education and training of future doctors

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INTRODUCTION

Nikolai Pirogov (1810–1881) is one of the most prominent representatives of medicine of the nineteenth century. Indeed, he was a comprehensively educated surgeon, physician, anatomist, and pathologist, as well as and the founder of field surgery and topographic anatomy, pioneer of plastic surgery, a renowned teacher, innovator, and reformer of Russian education, both secondary and university. By his contribution to medicine and pedagogy, Nikolai Pirogov, rightfully can stand alongside the polymaths of the Renaissance. The figure of N.I. Pirogov, his biography, various aspects of medical, surgical and pedagogical activity are constantly in the focus of scientific interests of many scientists, as evidenced by numerous research works [1; 2; 3; 4; 5; 6; 7; 8; 10; 11; 12].

THE AIM

The aim of the research is to analyze the scientific and pedagogical heritage of N.I. Pirogov through the prism of his outstanding polymathic abilities.

MATERIALS AND METHODS

Historiographical analysis is the main method used in this research. In addition to publications in which the biography of N.I. Pirogov was studied from different angles, the autobiographical work of the scientist «From the diary of an old doctor» and his epistolary inheritance were analyzed. We also used general scientific methods of analysis, synthesis, and generalization.

REVIEW AND DISCUSSION

Childhood and youth. Nikolai Ivanovich Pirogov was born on November 25, 1810 in Moscow. He was the penultimate of fourteen children (and one of the six who survived) of Ivan Ivanovich Pirogov, treasurer of the Moscow Provincial Depot. Nikolai Ivanovich's grandfather came from peasants and served as a coachman. Pirogov received primary education at home. N. Pirogov wrote that the first teacher started to teach him during the ninth year of his life, and before that, he was self-educated, with the help of his mother and sisters. His first teacher was a university student, and his second teacher was a student from Moscow Medical and Surgical Academy. At that time, young Pirogov already read and translated into Latin quite well [9, p. 444]. As a child, Pirogov already aspired to become a doctor. He described his «playing a doctor» as follows: «having seated several people, including a cat, dressed as a lady, passing from one patient to another, I sat at a table, wrote prescriptions, and kept reading how to take medicine» [9, p. 458]. Subsequently, Dr. G. Berezkin presented Nikolai with «Directory of plants used in medicine», and the boy first began to collect herbarium, and then created his own «herb collection» (the so-called book describing medicinal plants and methods of treatment with these plants) [7].

Pirogov (together with his brother Amos) received secondary education at a private boarding house of Vasiliy Kriazhev, but since his father bankrupted – Ivan Pirogov's subordinate stole a large sum of money and fled to Moscow, there were no money to pay for Nikolai's further education. According to advice of Professor Yefrem Mukhin, who at that time was the Dean of Medical Faculty and was con-

sidered one of the best practitioners in Moscow (he was invited to treat Pirogov's elder brother, who was ill with rheumatism but, unfortunately, died of the disease at a young age), 14-year-old Nikolai writes an application for admission to Moscow University, successfully passes the exams and becomes a medical student.

The Dörpt periods and internships in Europe. In 1828, after graduating from Moscow University, N. Pirogov (at that time a young doctor, aged 17), was sent to Dörpt (now Tartu, a city in Estonia) to study at the Professor's Institute in order to prepare for teaching surgery and invasive arts. Professor J. Moier (1786–1858) immediately drew attention to the extraordinary abilities and hard work of the young doctor, who began to engage N. Pirogov in his scientific activity. In addition, they socialized informally – the professor invited Nikolai Ivanovich to his weekends, together they had lunches and discussed the latest scientific achievements, and subsequently Pirogov almost became a member of J. Moier's family and visited his house almost daily [7; 9]. In December 1829, N. Pirogov won a gold medal for the competition work «What is observed in the ligation of the large arteries?».. On August 31, 1832 (he was only 22 years old at that time) he defended his thesis «Num vinctūra aortae abdominālis in aneurysmăte inguināli adhibitu facĭle ac tutum sit remedium?» («Is bandaging of the abdominal aorta with inguinal hernia an easy and safe intervention?»). In this work, N. Pirogov investigated and described the location of the abdominal aorta in humans, circulatory disorders in its dressing, the ways of circulation in the obstruction of this aorta, and explained the causes of postoperative complications. He also offered two accesses to the aorta: abdominal and extra-ventricular. The dissertation was of great theoretical and practical importance, since before N. Pirogov the ligation of the abdominal aorta, followed by the *exĭtus letālis* of the patient, was performed only once – by an English surgeon, court physician of the kings George IV and William IV, as well as Queen Victoria, Sir Astley Paston Cooper (1768–1841) [7]. In 1833–1836, N. Pirogov was in Germany – Berlin and Goettingen, where he was acquainted with the experience of teaching surgery. During this period, a great influence on the further development of Pirogov as a scientist and practicing surgeon was exerted by professor of the University of Göttingen, surgeon Bernhard Rudolf Konrad von Langenbeck (1810–1887), professor of the University of Berlin, anatomist F. Schlemm (1795–1858), and a prominent physiologist, specialist in comparative anatomy, ichthyologist and herpetologist Johannes Peter Müller (1801–1858). Undoubtedly, Professor Langenbeck, who taught Pirogov the purity of surgical techniques, taught him «to hear the completed melody of surgery», had the greatest influence on the young N. Pirogov, showed how it was necessary to adapt the movements of the legs and the whole body to the movements of the hands during surgery. The most talented student, N. Pirogov, became the embodiment of an ideal surgeon, according to Langebeck, who despised slowness and immobility and demanded clear and harmonious work from his students [7, p. 207].

German scientists highly appreciated the scientific achievements of young Pirogov, in his memoirs, he wrote that the famous Opitz got acquainted with his thesis and immediately ordered to translate it from Latin into German and print it in the then famous Grefe and Walter journal, and the findings and recommendations by Pirogov began to be actively implemented by many surgeons in different corners of the world. Returning home from Germany, Pirogov came down with typhoid fever and was forced to stay in Riga for further treatment, where, after recovery, he began his surgical career. His career in rhinoplasty began: N. Pirogov provided a noseless barber with a new nose, which he would later call the «best made nose» in his life. From Riga, N. Pirogov went to Dörpt, as he learned that the Moscow department which he had been promised to head, was given to another contender – F. Inozemtsev (1802–1869). N. Pirogov himself wrote about this fact on December 27, 1880, to Y.V. Bernstein [9, p. 429]: «while I was lying sick in the Riga military hospital, Inozemtsev, my friend, was elected... as a Professor of Surgery in Moscow, and I, a Muscovite, remained no beans to count and in a hospital bed» [9, p. 431]. However, N. Pirogov was given a department in Dörpt, which was handed to him by his former mentor – Professor J. Moier. N. Pirogov's Dörpt period lasted for five years. The great scientist wrote about these years as follows: «…5 years of professorship, which required daily eight-hour sessions at the clinic, lecture room and anatomical theater with attendees» [9, p. 431].

During these five years, N. Pirogov published works that have not lost their scientific significance even now: 1) «Anatomia chirurgĭca truncōrum arterialium atque fasciārum fibrosārum» («Surgical Anatomy of Arterial Trunks and Fibrous Fascia», 1837–1838), in Latin and German with atlas *in folio*; 2) «Annals of the Surgical Clinic», 1836–1837; 3) «Clinical Annals», 1837–1838; 4) the monograph «On the Achilles Tendon Insicions». Undoubtedly, the most outstanding of these works was «Anatomia chirurgĭca truncōrum arterialium atque fasciārum fibrosārum», in which N. Pirogov laid the foundations of surgical anatomy – a science, which he created step by step, and for which he was awarded the Academic Demidov Prize in 1840.

The Petersburg period. In 1841, N. Pirogov was invited to become the head of the Department of Surgery, Pathological and Surgical Anatomy at St. Petersburg Medical and Surgical Academy. Last but not least, this was made possible thanks to the project of introducing hospital classes in Russia for graduates and young doctors, which was submitted by Pirogov to the Trustee of this institution – Kleinmichel. The project was accepted and approved, and N. Pirogov himself headed the Department of Hospital Surgery at the 2nd Military Land Hospital, and also embraced the position of the senior doctor of the surgical department at the same hospital with the right to attend to patients (i.e., the thoracic department). The undeniable merit of N. Pirogov is that since then the hospital clinics have gradually become established at all Russian universities.

N. Pirogov worked at Medical-Surgical Academy for 14 years – these were the years of the height of his talent

and his self-fulfillment as a scientist, teacher and practical surgeon. In the letter of Y.V. Bernstein as of December 27, 1880, Pirogov wrote that for 14 years, he not only taught the course of pathological anatomy, but also personally kept the protocols of 11000 autopsies [9, p. 429]. N. Pirogov was an excellent lecturer. According to contemporaries, his lectures, which were distinguished by a clear manner of teaching and extraordinary content, always took place in overcrowded lecture theatres [2]. Journals and newspapers wrote about N. Pirogov, and his lectures on surgical dissections, sutures, purulent inflammations and autopsy results were metaphorically compared to the performance of the Italian opera singer Angelica Catalani, highly popular at that time – a phenomenal soprano of extremely beautiful and pure timbre, taking G in the third octave.

In 1846, Pirogov was again sent abroad to become acquainted with the organization of anatomical institutions. Since N. Pirogov's duties included the training of military surgeons, he not only began to study the common surgical methods at the time, but also radically refined and improved many of these methods and techniques. Hence, Pirogov paid much attention to the introduction of anesthesia (etherification) into surgical practice. The result of this was his work «Etherisation per rectum», written in French and published in 1847. In July of the same year, during the next Caucasian War, N. Pirogov performed the world's first field operation using anesthesia *per os et per rectum*. He also introduced such new methods of anesthesia as the intravenous and intratracheal routes. During the same military campaign, N. Pirogov for the first time applied fixed bandaging, impregnated with starch, to immobilize complex limb fractures, known as «Seutin bandage» (Louis Joseph Ghislain Seutin, 1793–1862, a Belgian surgeon who suggested this method of immobilization). During his stay in the Caucasus and southern Russia, on behalf of the military department, Pirogov inspected more than 100 military hospitals and in 1848, upon returning to St. Petersburg, issued a «Report on a Medical Trip to the Caucasus», accompanied by an atlas, statistics of all operations performed by him, the use of anesthesia and his own studies of gunshot wounds, which he studied in the wounded and in corpses [p. 432].

In the summer of 1848, an epidemic of cholĕra Asiatĭca began in St. Petersburg, and Pirogov organized a special ward for patients at his hospital clinic. The scientist wrote that within 6 weeks he had autopsied 600 deceased with this diagnosis. Furthermore, he presented the results of his own research in the book «Pathological Anatomy of Asiatic cholera» in Russian and French with atlas *in folio*. For this research, the scientist was awarded the Great Demidov Prize from the Academy of Sciences. The period of 1849–1852 was also very fruitful. During these years, N. Pirogov continued to be active in teaching activities, he also headed the Anatomical Institute, consulted patients in five hospitals: Obukhovskaya, Mary Magdalen, Petropavlovskaya, Children's and Masimilianova. In addition, he worked on «Annals of Hospital Clinic», where he gave a description of an osteotomic amputation at the ankle joint, and the book «On Happiness in Surgery», which he wrote in German.

During the Caucasus Campaign of 1851 – 1852, Pirogov worked on improving the immobilizing starch «Seutin bandage», as previous experience showed its shortcomings in the practice of field surgery. In this regard, he began developing the plaster bandage. The scientist himself wrote as follows: «…I undertook the application of my fixed bandage on the battlefield, because from the experience of the siege of Salta (this is the first Caucasian experience of N. Pirogov – military operation near the village of Salta – the territory of modern Nagorno-Dagestan; the siege of this the village lasted 54 days – from July 25 to September 15, 1847, and Pirogov was directly involved in the military conflict as a surgeon) I saw the various disadvantages of the Seutin starch band… once I went to the sculptor, and became acquainted with the plaster casting of the canvas and immediately applied and tested plaster bandages at hospitals, being sufficiently convinced of their convenience» [9, p. 433].

At about the same time, in search of an effective method of teaching surgery, Pirogov decides to organize anatomy training on frozen corpses (the scientist himself referred to this method as «ice anatomy»). The result of this innovative anatomy study was the publication of the unique anatomical atlas «Anatŏme topographĭca sectionĭbus per corpus humānum congelātum triplĭci directiōne ductis illustrāta» («Topographic Anatomy: Illustrated Autopsy, Conducted through the Frozen Body of a Man in Three Directions», 1851–1859) – it was an edition containing 995 full-size cross sections (like in a 3D format) with four notebooks of various prefaces and detailed explanations. Thanks to this atlas, surgeons were able to perform operations with minimal injury to patients. Nikolai Ivanovich wrote about his works without undue modesty: «My works for the first time showed with accuracy and clarity the relation of the fascia to the arterial trunks and indicated the ways most convenient and accurate to perform surgery on the arterial trunks. The sections of organs and cavities frozen in different positions together with the anatomical sculpture provided a way to determine the normal anatomical position and the interposition of different organs and joints with accuracy, impossible through the usual way of study» [9, p. 437]. Moreover, the cross section method proposed by Pirogov became the basis for such extremely common and informative methods of life-time diagnostics as computed tomography (CT) and magnetic resonance imaging (MRI). N. Pirogov participated in the Crimean campaigns of 1853 and 1855. Thus, during the Crimean War of 1853 he was the chief surgeon besieged by the Anglo-French troops of Sevastopol. During this military campaign, N. Pirogov, operating the wounded, for the first time in the history of medicine, used a plaster band to immobilize the limb, thus establishing the tactics of preserving the injured limbs and saving many soldiers from amputation. During the siege of Sevastopol, Pirogov supervised the nurses' training and work – the Holy Cross community of sisters that was an unprecedented innovation until then. N. Pirogov is con-

sidered the founder of military surgery, because it was he who introduced a new method of care for the wounded: the injured were subjected to careful examination and triaging at the first dressing point. Depending on the severity of injuries, some had to be operated as quickly as possible in the field, while others, with minor injuries, had to be evacuated deep into the country for treatment in stationary military hospitals.

In 1861–1864, a book by N. Pirogov, «The Basics of General Military Field Surgery», was published in Leipzig in German, where the scientist presented his views on hospitals, medical administration, dressing points and wound healing. Nikolai Ivanovich wrote about this work: «My proposed system of triaging the wounded and the energetic resistance against the evil inflicted on the wounded at hospitals made a great impression. This book represented the ideal of the Red Cross Association before it was officially adopted.

Personal life. N. Pirogov was quite uncompromising in his personal life. In the second year of his stay in St. Petersburg, he became ill and thought that he had no family, so he finally decided to marry. The great scientist was married twice: for the first time – to Katerina Dmitrievna Berezina (1822–1846) – a girl from the noble but impoverished family (she was the granddaughter of the famous Earl M. Tatishchev – cavalier of all orders of the Russian Empire, her father gambled away her mother's dowry). According to biographers, the wedding, which took place on December 11, 1842, was quick and modest. During the fourth year of married life, Katerina died of complications during childbirth, leaving Pirogov with two sons: the elder – Nikolai (1843–1891) and the younger – Vladimir (1846–1910). With all his frankness, N. Pirogov confessed that after the death of his wife, he felt very lonely – two attempts to marry were not successful. Meanwhile, few friends have told Nikolai Ivanovich about a girl who is passionate about reading his article about a perfect woman. Four years after the death of his first wife, Pirogov married for the second time. His second wife and, as it turned out later, the love of a lifetime, as well as a loyal friend and soulmate, was that very girl who enthusiastically read the article about a perfect wife – Baroness Alexandra Antonovna von Bistram (1824–1902) – the daughter of General Lieutenant A. von Bistram and the great-granddaughter of the navigator I.F. Krusenstern. N. Pirogov had no children from this marriage.

Vinnytsia period. In 1859, shortly before his retirement, N. Pirogov bought an estate with the romantic name «Vyshnia» (meaning «Cherry») near Vinnytsia, where his family settled. In his estate, N. Pirogov organized a charge-free hospital. After his resignation, the scientist traveled abroad several times on behalf of the Red Cross, and also to St. Petersburg to lecture at the invitation of St. Petersburg University. During the Franco-Prussian War, the scientist was invited to inspect the military hospitals opened on the frontline. N. Pirogov inspected more than 70 infirmaries and was widely respected everywhere, because the vast majority of Prussian doctors applied the techniques and relied on the principles of military surgery, established down by this distinguished scientist. The inspection resulted in the «Report on Visits to Military Establishments in Germany, Lorraine and Alsace», published in Russian and German. Like anything done by N. Pirogov, this report was of great importance for the improvement of medical treatment in the army and organization of first aid to the wounded [6]. As one of the most famous surgeons of the nineteenth century, N. Pirogov treated German Chancellor Otto von Bismarck, the Russian composer Peter Tchaikovsky, and also saved the leg of Giuseppe Garibaldi, the Italian national hero, commander, revolutionary and political figure, from amputation [1].

Illness and death. At the beginning of 1881, N. Pirogov drew attention to the pain and irritation of the mucous membrane of the hard palate. On May 24, the same year, the eminent surgeon M. Sklifosovskyi (1836–1904) diagnosed N. Pirogov with cancer. The caring wife first took Pirogov to Moscow, where he was examined by local physicians, and then to Vienna for consultation with the well-known surgeon Billroth, who assured Nikolai Ivanovich that the ulcer on the palate was benign and the operation was unnecessary... A prominent surgeon died of cancer on 5 December (23 November) 1881. According to the legend, during the agony of Nikolai Ivanovich, a lunar eclipse occurred. N. Pirogov's widow received permission from the Orthodox Church to embalm the body of the prominent surgeon, after which it was moved to the basement with a window, over which the Church of St. Nicholas was built in 1885. Until her death (1902), Alexandra Antonovna took care of the estate, but due to material difficulties that occurred in the family of the great surgeon after his death, the estate gradually declined. Around 1914, the grave of a great scientist and doctor was devastated: an épée – a gift from the Austrian Emperor Franz-Joseph, was stolen from it, as well as a metal cross lying on Pirogov's chest. The further fate of these valuables is unknown. During the grand opening of the monument to Pirogov, another prominent surgeon in Moscow, M. Sklifosovskyi, delivered a speech in which the essence of Pirogov's figure is concentrated: «Pirogov's contribution to science… will remain an eternal treasure and cannot be erased from the historical tablets until the European science exists… The people, who had their Pirogov, have the right to be proud…» [11].

CONCLUSIONS

The authors analyzed the epistolary heritage of N. Pirogov, which served as a valuable biographical source. Given the anthropocentrism of the current stage of the existence of society and European civilization, the article sought to "revive" the biography of N. Pirogov. In this regard, considerable attention is paid to his personal life. The scientific novelty of the research is that the biography of N. Pirogov is represented with refinements and additions based on his latest work "From the Diary of an Old Doctor". As a result, the article considers this outstanding personality in a new perspective, presenting the main stages of his scientific and medical activity. The study of the scientific and pedagogical

heritage of N.I. Pirogov is a basis for generating new ideas and developments in the modern medical education, as a classic example of combination of professional and intellectual, theoretical and practical, social and personal skills and abilities of a doctor.

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CASE STUDY

DEVIC'S OPTICOMYELITIS: A CASE REPORT FROM THE AUTHORS' CLINICAL PRACTICE

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ABSTRACT

The aim was to analyze the contemporary scientific literature on Devic's opticomyelitis and to present a case report from our clinical practice.

Based on the patient's complaints, case history and features of clinical course, objective neurological status, clinical laboratory and additional examination methods, characteristic MR-patterns, consultations of related specialists and differential diagnostics, we made the clinical diagnosis according to ICD-10: G36.0 Devic's opticomyelitis, exacerbation, with a sustained bilateral lesion of the optic nerves in the form of retrobulbar neuritis with the development of partial atrophy of the optic nerves in both eyes, spinal cord lesions with common cystic, cicatrical and atrophic alterations at C1-Th8 level with moderate lower paraparesis, expressed by sensory ataxia, sensory disturbances by the descending conductive type from Th10, impaired function of pelvic organs by the type of acute urinary retention, asthenic and neurotic syndrome.

Widespread cases of demyelinating pathology in medical practice and complexity of differential diagnostics determine the need for a specific diagnostic algorithm. This algorithm should consider anamnestic data along with the course of the disease, clinical, laboratory and instrumental examination, including neuroimaging, analysis of CSF for oligoclonal bands, analysis for IgG antibodies to AQP4, which will allow to carry out diagnostics and to decide on tactics for further management of patients of this cohort. Further research is needed to conduct additional studies for optimization of tactics for dynamics monitoring and improvement of diagnostic, treatment and rehabilitation measures in patients with Devic's opticomyelitis, including appropriate immunological control, given the complexity of differential diagnostics and the affinity of this pathology to multiple sclerosis.

KEY WORDS: Devic's opticomyelitis, diagnostic algorithm, differential diagnostics, treatment, aquaporin-4, IgG antibodies

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INTRODUCTION

Opticomyelitis (neuromyelitis optica, Devic's syndrome / disease) is a severe idiopathic demyelinating disease of the central nervous system, characterized by the prevailing lesion of the optic nerves and spinal cord with relative intactness of brain structures [1]. For a long time, this disease was considered within the framework of malignant variants of multiple sclerosis. However, the progress in the pathogenesis study on demyelinating diseases of the central nervous system (CNS) at the end of the 20th century allowed the researchers to distinguish opticomyelitis as a separate nosological form [2].

CASE REPORT

The aim of the research was to analyze the contemporary scientific literature on Devic's opticomyelitis and to present a case report from our clinical practice.

Opticomyelitis is a disease, which is autoimmune by nature. The ratio of affected women to men is (2-8):1. The age of the disease onset varies from 1 to 77 years, most often starting at the age of 35-47 years, more commonly in the non-Caucasian representatives [3; 4]. The pathogenesis of the disease is based on the formation of NMO-IgG autoantibodies to aquaporin-4 (AQP4) (the protein of water-conveying canals of cell membranes), which localizes at the peduncles of astrocytes forming the blood-brain barrier. The highest concentration of AQP4 in the CNS is observed in the gray matter of the spinal cord, hypothalamus, periventricular areas [5].

Therefore, the foci in the brainstem and hypothalamus may be considered relatively characteristic and specific; the cerebral foci by their localization tend to those areas of the brain that display a high level of immunoreactivity to AQP4 [6]. Pathophysiologically, demyelination and necrosis of the white and gray matters occur in opticomyelitis. Chronic foci of inflammation in the brain are represented by cystic degeneration, gliosis, and nerve atrophy, which can lead to the development of secondary syringomyelia [7]. Initially, in the clinical presentation of the disease, there is visual impairment in the form of reduction, until its complete loss, and after a while the symptoms of severe transverse myelitis develop – para- and tetraparesis, impaired function of the pelvic organs. It is now assumed that opticomyelitis can have both a single-phase and a remittent type of the course; however, repeated attacks are less typical than remissions [8].

Optic neuritis is one of the main symptoms of opticomyelitis. During ophthalmoscopy, a normal pattern of the fundus is more often observed, a slightly blurred optic discs, slight edema, atrophy and pallor of the optic nerves in chronic cases. In opticomyelitis, optic neuritis is usually bilateral, commonly preceding myelitis (in 80% of cases). In a few weeks, less frequently in a few months, severe transverse myelitis develops, whose typical symptoms are muscle weakness, spasticity, discoordination, ataxia, Lhermitte's sign, urinary retention, autonomic dysfunction, possible sensory disorders below the level of the lesion of the spinal cord. In most cases, myelitis occurs less than 3 months later. However, in 20% of cases, transverse myelitis may precede optic neuritis [9].

Changes in the optic nerves can be detected by neuroimaging, since they involve the optic nerves over a greater length, unlike changes in multiple sclerosis. In almost all cases, opticomyelitis has to be differentiated from multiple sclerosis. In opticomyelitis, either brain MRI does not reveal any pathological changes, or in almost half of cases there are non-specific, often asymptomatic, foci of demyelination. On MRI in multiple sclerosis, foci in the spinal cord usually do not exceed one segment in length, whereas in opticomyelitis, foci exceeding three or more segments are visualized.

The analysis to determine IgG antibodies to AQP4 is diagnostically important. Additional features are the results of CSF analysis, its study for the presence of oligoclonal bands. In 2008, the international work group reviewed and formulated the diagnostic criteria for optic neuromyelitis (by D.H. Miller et al., 2008): 1) the "major" criteria (the presence of all essential criteria is required, with an indefinite time interval between them): optic neuritis with the lesion of one or both eyes; transverse myelitis, clinically complete or incomplete, but associated in the acute period with the presence of radiologically confirmed lesion of the spinal cord that extends longer than 3 segments on T2-weighted MRI images and is hypo-intensive on T1-weighted images; lack of data on sarcoidosis, vasculitis, systemic lupus erythematosus, Sjogren's syndrome, or other explanation for the disorder; 2) the "minor" criteria (at least one must be relevant): the latest brain MRI should display no pathology or detect only pathological changes that do not meet Barkoff's criteria as reflected in McDonald's criteria (2005); positive serum or cerebrospinal fluid test for NMO-IgG / antibodies to AQP4.

In terms of therapeutic tactics, there is currently no common standard in the treatment of this pathology. Symptomatic and restorative therapies are used to support the existing neurological functions. For the treatment of myelitis and optic neuritis attack, high doses of corticosteroids are used (methylprednisolone 1000 mg daily intravenously No.5 consecutively), then it is recommended to administer prednisolone maintenance therapy at a dose of 1 mg / kg / day as part of initial immunosuppressive therapy to prevent recurrent attacks [10]. Unfortunately, myelitis often poorly yields to such therapy, and sometimes it is even aggravated. In these cases, plasmapheresis is recommended (seven sessions a day, in 55 ml / kg per metabolic transfusion) [11]. For long-term treatment of Devic's opticomyelitis, it is recommended to apply immunomodulatory therapy, rather than the immunosuppressive one. Most practitioners consider the combination of oral prednisolone and azathioprine to be a therapy of choice, followed by gradual reduction of corticosteroids to the lowest effective dose or their complete withdrawal and azathioprine monotherapy [12]. Despite therapy, Devic's opticomyelitis in some cases leads to lethal outcome, often as a result of severe attack of myelitis with involvement of the cervical spinal cord and development of respiratory disorders [13].

Case presentation: Patient O., born in 1995, in March 2018 presented with urinary disorders (urinary retention), weakness in the legs, numbness and impaired sensitivity in the legs and the lower body, grogginess when walking, decreased vision, pain in the thoracic spine, excessive fatigue and severe weakness. The patient considered herself ill since December 2015, when she had noticed a sharp deterioration of vision in the right eye. She had been treated at the ophthalmology department of the regional hospital (retrobulbar neuritis on the right) with a positive dynamics. In May 2017, she again had suffered from visual impairment in the right eye. The patient had undergone MRI of the brain with intravenous contrast (MR signs of the focal lesions of the spinal cord at the level of C2-C3, the right optic nerve, probably of demyelinating character (Fig. 1, 2)), pulse therapy with corticosteroids (methylprednisolone 1000 mg intravenously, by drop infusion No.5) with a tendency to positive dynamics, but in 2 weeks there had been a decrease in vision in the left eye as well. Plasmapheresis course had been conducted at the end of May 2017, and there had been a slight positive dynamics. In August 2017, the condition had aggravated again: visual impairment and sensitivity in the legs and lower left trunk with weakness in the left leg had developed. MRI of the brain, cervical and thoracic spine had been performed (no data on the volumetric, focal processes of the brain had been detected; MR signs of demyelinating changes in the spinal cord at the levels of C1-C3, C7-Th4, Th7-Th8 (Fig. 3)), the course of pulse therapy with corticosteroids (methylprednisolone 1000 mg intravenously by drop infusion No.5) and neurometabolic therapy had been conducted with a positive dynamics. It was also known that the patient had been periodically self-treated with non-steroidal anti-inflammatory drugs for pain in the spine, mostly in the cervical and thoracic regions. Marked deterioration of the condition had been observed about 3 days before addressing a doctor after contracting acute respiratory viral infection with hyperthermia, when there had been a significant increase in leg numbness, weakness, and urinary retention had joined the abovementioned complaints.

At the time of admission: skin and visible mucous membranes were pale pink; blood pressure was 115/70 mm Hg, heart rate was 74 beats per minute; heart tones were rhythmic, sound; abdomen was soft on palpation, sensitive in the lower parts, the bottom of the bladder was determined by palpation and percussion – urinary retention. Neurological

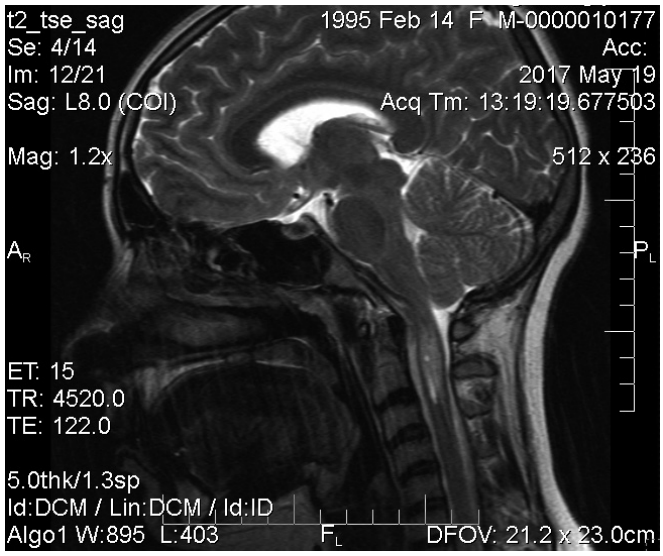

Fig. 1. MRI of the brain of patient O. (May 19, 2017)

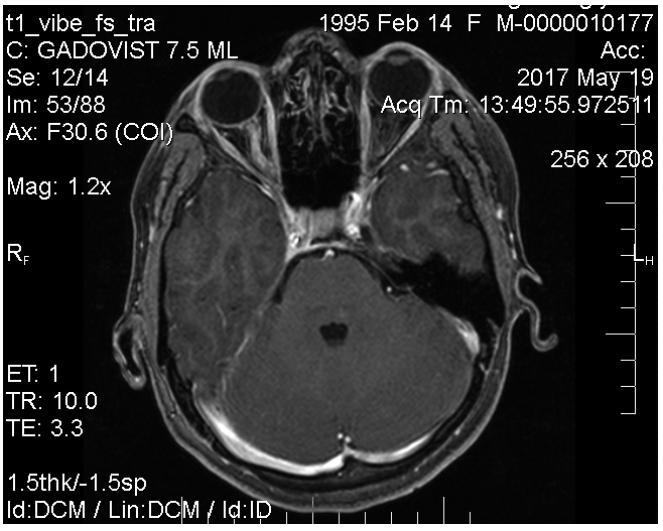

Fig. 2. MRI of the brain of patient O. (May 19, 2017)

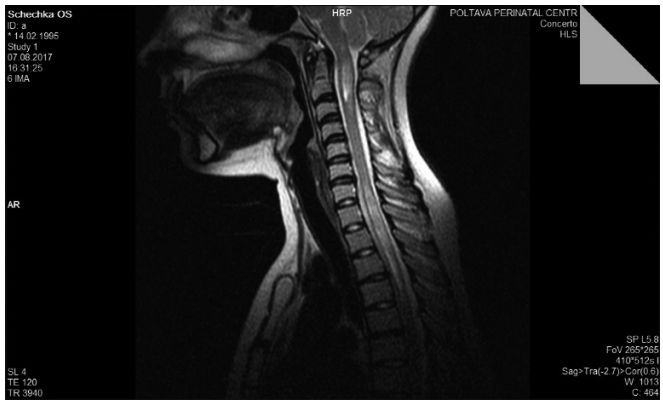

Fig. 3. MRI of the cervical and thoracic spine of patient O. (August 07, 2017)

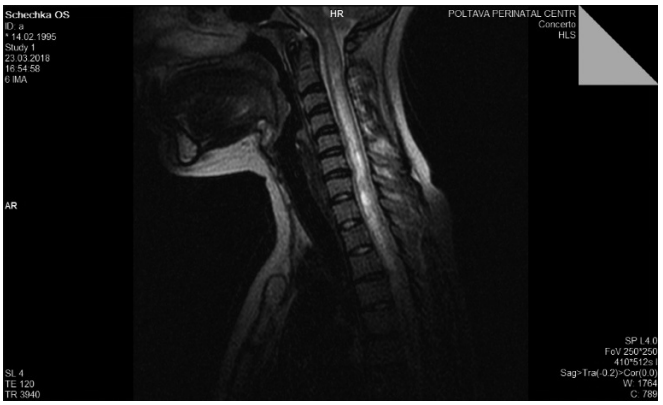

Fig. 4. MRI of the cervical and thoracic spine of patient O. (March 23, 2018)

status: palpebral fissures D=S, pupils D=S. Photoreactions were preserved. There is no nystagmus. The exit points of the V pair were painless. The face was symmetrical, the tongue was along the middle line. Reflexes from the back of the pharynx and soft palate were preserved. Swallowing was not impaired, the voice was loud. The speech was preserved. The muscular tone was dystonic in hands, and increased by spastic type in feet. Barré test was "+" in the legs. The strength in hands was retained, and reduced in the legs to 3.0 points. Babinski's symptom was (+) on two sides. Hand reflexes D=S were high, with extended reflex zones, abdominal abs reflexes, knee reflexes D=S, high, Achilles reflexes D=S, polykinetic. There is a pronounced descending hypesthesia by the conductor type from Th10 with gross disturbance of vibration sensitivity in the legs. There were no meningeal signs. Coordination tests were performed with ataxia in the lower extremities. The patient was anxious, with distal hyperhidrosis, hypothermia; dysfunction of the pelvic organs by central type (urinary retention).

The comprehensive clinical laboratory and instrumental examination was conducted along with consultations from related specialists (urologist – acute urinary retention; ophthalmologist – partial atrophy of the optic nerves in both eyes), which allowed us to confirm the absence of systemic vasculitis, other rheumatological and infectious pathologies. During the examination, the following results attracted special attention and served as a confirmation of the correctness of our diagnostic search: analysis for IgG antibodies to AQP4 (21.03.18): 1:320 (positive result); MRI of the brain, cervical and thoracic spine with intravenous contrast (23.03.18): no data on volumetric, demyelinating processes of the brain were found, the effects of previously sustained myelitis with extensive cystic, cicatrical and atrophic changes at the level of C1-Th8. There has been a negative dynamics as compared to MRI as of August 2017.

Based on the patient's complaints, case history and features of clinical course, objective neurological status, clinical laboratory and additional examination methods, characteristic MR-patterns, consultations of related specialists and differential diagnostics, we made the clinical diagnosis according to ICD-10: G36.0 Devic's opticomyelitis, exacerbation, with the sustained bilateral lesion of the optic nerves in the form of retrobulbar neuritis (May 2017) with the development of partial atrophy of the optic nerves in both eyes, spinal cord lesions with common cystic, cicatrical and atrophic alterations at C1-Th8 level

(according to MRI data as of 23.03.2018 (Fig.4)) with moderate lower paraparesis, expressed by sensory ataxia, sensory disturbances by the descending conductive type from Th10, impaired function of pelvic organs by the type of acute urinary retention, asthenic and neurotic syndrome.

The patient underwent a course of pulse therapy with corticosteroids (methylprednisolone 1000 mg intravenously by drop infusion No.5) with subsequent transition to oral methylprednisolone according to the scheme of gradual dose de-escalation, angio-, neuroprotective, antioxidant and physiotherapy. The patient refused from therapy in combination with azathioprine. During inpatient stay, no intolerance to medicines was observed. As a result of the conducted treatment, a positive dynamics was observed: urination resumed (acute urinary retention regressed to the neurogenic bladder), strength in the legs increased (moderate lower paraparesis regressed to the mild one), ataxia and general weakness decreased, sensitivity resumed in the lower part of the trunk and improved up to the level of the upper third of the thighs.

CONCLUSIONS

Thus, taking into account the widespread cases of demyelinating pathology in medical practice, and keeping in mind the cases of Devic's opticomyelitis and complexity of their differential diagnostics, requiring clear clinical thinking, the necessity to follow a specific diagnostic algorithm becomes obvious. This algorithm should consider anamnestic data along with the course of the disease, clinical, laboratory and instrumental examination, including neuroimaging, analysis of CSF for oligoclonal bands, analysis for IgG antibodies to AQP4, which will allow to carry out diagnostics and to decide on tactics for further management of patients of this cohort. Further research is needed to conduct additional studies for optimization of tactics in dynamics monitoring and improvement of diagnostic, treatment and rehabilitation measures in patients with Devic's opticomyelitis, including appropriate immunological control, given the complexity of differential diagnostics and the affinity of this pathology to multiple sclerosis.

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CASE STUDY

OCCLUSAL TRAUMA OF IMPLANT-SUPPORTED METAL-CERAMIC CROWN: A CASE REPORT

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ABSTRACT

In this article there is a clinical case of occlusion trauma of implant-supported metal-ceramic crown for prosthetics of central incisor. Its uniqueness is the possibility to save dental implant after acute occlusion impact, which was strong enough to break ceramic facing of fixed denture, but not able to destroy bone and implant components. The occlusion force located at the incisal edge of the crown induced a reverse torque to the implant and did not result in its failure or bone resorption. In a year after repeated fixed prosthetics, the results of clinical examination proved absence of any problem with osseointegration. Literature analysis lets us to suggest, that the phenomenon was caused by protective action of cortical bone around of dental implant. Besides, in the case of natural tooth, the bone is suddenly compressed against the conical root; it transfers occlusal breaking to the supporting periodontal ligament. A dental implant has no periodontal ligament but can have a rough surface that may preclude implant failure.

KEY WORDS: dental implant, fixed denture, occlusal trauma

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INTRODUCTION

Initial stability of dental implant is an important factor of prosthetic success. According literature analysis, immediately loaded implant has micro-motion of less than 150 pm. So, it is still osseointegrate; excessive micro-motion causes fibrous encapsulation [1]. However, the more significant trauma for a dental implant is an occlusion load. But the process of osseointegration depends not only on character of occlusion but on density of bone tissue, surface texture, diameter and length of the implant [2-4].

The occlusal trauma damages osteocytes and cellular structures that are in close proximity to implant surface and create a space between the implant and bone for epithelial down-growth. Occlusion forces in the lateral parts of dentitions are about 120 and 150 N. Occlusal load of less than 200 pm shows no significant increase of implant load level. Thus, these occlusal forces may not cause failure of integrated implants if they do not induce a micro-movement of more than 150 pm [5]. The most dangerous occlusion forces are chronic, severe, and variable in magnitude, with different eccentric direction and frequency [6-7]. In turn, short implants can be used for anchorage for the small, unidirectional and constant forces for orthodontic tooth movement [8].

The interaction of bone and implant interfaces under an occlusion force is unknown. A study in rabbit femurs showed that the torque removal force of implants ranged from 27 to 59 N at 3-month post-insertion [9]. Titanium oxide layer thickness, micropore configurations and its crystal structures apparently affect bone tissue response. 600-1000 nm of oxide layers demonstrated significantly stronger bone responses in evaluation of removal torque than implants with an oxide layer less than 200 nm [10].

It has been suggested that a placement torque force of greater that 42 N /cm allows a newly installed implant to be immediately subjected to non-functional load. That is, a newly placed single implant can be fitted immediately with a provisional crown but so as to be out of the path of movement of the opposing teeth [11].

In these cases, the occlusal forces of the opposing dentition are not in direct contact with the new crown and implant complex. The forces to bear are usually those of the soft tissues of tongue and mucosa and compressed food boluses from mastication. The bone around the newly placed implant is probably able to resist these lesser forces and would not move the newly placed implant more than 150 pm in the required rigid bone that encases it [12].

The immediate placement and functional (occlusion) load is possible with cross-arch stabilization, but the survival rate is 85%. Implant immobilization brought with the arch form of denture distributes occlusal load. This arch form distribution and multiplicity of implants probably prevents any implant movement beyond 150 pm [13].

The dental implants located in the anterior part of mandible can be placed in immediate true function and provide immediate retention for an overdenture [14]. In maxillary overdentures with four to six implants, the forces of occlusion can loosen component screws, but apparently do not readily cause implant loss in the short term [15].

Coming from all the above, we considered it worthwhile to consider to the following case from our practice. It seems to be interesting because of abnormal occlusion load (trauma) in contrast to the literature data on physiological occlusion.

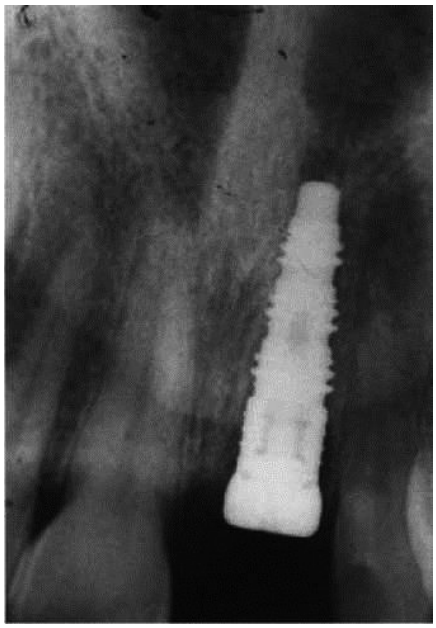

Fig. 1. Post-operative radiography

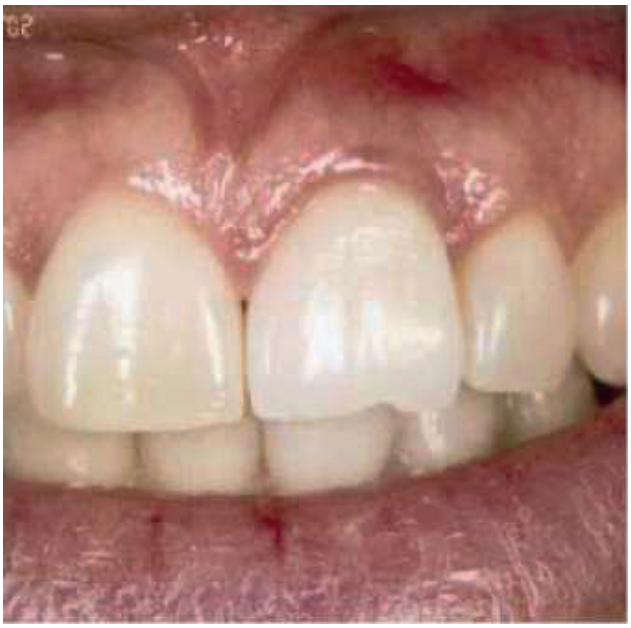

Fig. 2. Porcelain fracture and local gingivitis in 2 days after trauma

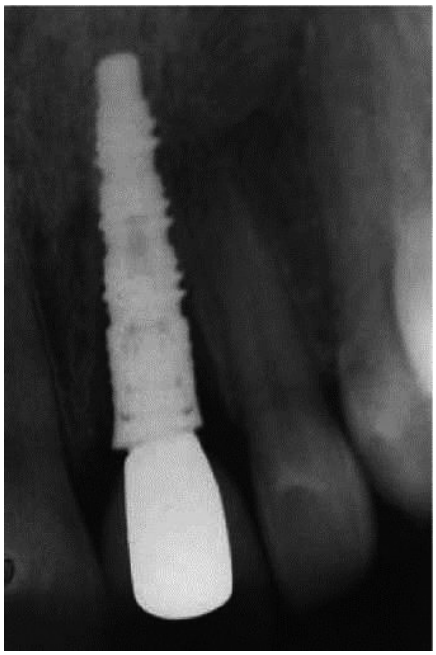

Fig. 3. Radiography in 2 days after trauma

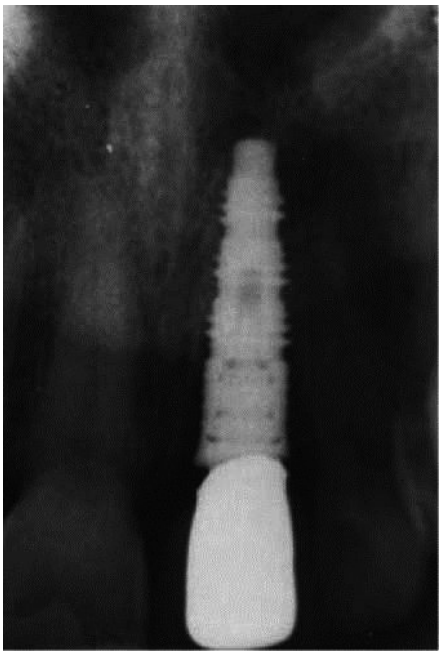

Fig. 4. Radiography in 5 months after trauma

CASE REPORT

A 25-year-old woman was indicated extraction of tooth 21. Endodontic and full crown treatments were failed; so, the tooth was not restorable. The tooth was extracted and intraosseous implant was immediately placed. A 1-mm gap at the lingual surface was filled with a bioactive glass ceramic (Fig.1).

A provisional removable denture replacing 21 was adjusted, relieved over the implant site and delivered. In half of a year, an abutment was placed, torqued into place and restored with a cemented metal-ceramic crown. The patient was satisfied with the results of prosthetics. But in a week, the patient needed for emergency treatment with a complaint of a fractured 21 crown and implant displacement. In anamnesis, two days before her visit, she had occlusion trauma to the implant crown. Any pain was absent. Objectively: the crown was mobile; it had fracture of ceramic on the cutting edge. The crown was in its original position. The gingival margin was a little swelling and hyperaemic (Fig. 2).

The implant was removal and no longer integrated (Fig. 3).

The patient was prescribed anti-inflammatory and antimicrobial treatment.

The crown was cut and removed from the abutment. The implant was found to be immobile, apparently still

integrated and undamaged. The abutment retaining screw had apparently loosened from the occlusion trauma. They were removed, inspected, found to be undamaged and replaced to their original positions. The abutment screw was again seated with a torque wrench. A new crown was made and cemented.

The implant has not exhibited any adverse effects from the trauma and there has been no unusual bone loss or other problems (Fig. 4).

When trauma of natural tooth occurs, the bone is suddenly compressed against the conical root and the tooth is propelled occlusal breaking the supporting periodontal ligament. A dental implant has no periodontal ligament but can have threads and a rough surface that may preclude avulsion.

A tooth that is luxated may fully recover by temporarily splinting the injured tooth to its neighbours for support for healing. The treatment for a mobile implant is probably removal. A dis-osseointegrated implant may develop infection, fibrous encapsulation or epithelial down-growth.

Natural teeth that are fractured can be restored unless a root fracture necessitates extraction. Apparently from the case now presented, an integrated implant fixture can sustain some magnitude of external trauma and survive.

It is possible that the bone around an implant can be destroyed. It seems that component parts loosen or fracture before the implant or the integrated bone housing fractures. Component screw loosening and fracture can occur before bone loss around implant fixtures restored in occlusal disharmony.

Thus, occlusal forces are variable, frequent, and multidirectional and increase the risk of failure, if non-axial. These occlusal forces always produce stress at the neck of an implant [16]. Axial forces produce the lowest stress. So, cclusal prosthetic design should, at best, prevent or minimize exposure to non-axial forces [17].

The molecular basis of the toughness and strength of bone is largely unknown. Bone is a nanocomposite of hydroxyapatite crystals and a collagen matrix. The crystals of hydroxyapatite cannot dissipate much energy from an impact, so the collagen matrix remains as the probable energy-absorbing entity. These bonds are thought to be responsible for the toughness of bone [18].

Microfractures of bone around implants are associated with oblique loads, high occlusal stress magnitudes and an absence of cortical bone [19]. Bone microcracks are precursors to fracture. The way bone is structured helps prevent crack initiation in transverse fracture under tension, shear and tear [20]. It can be that an osseointegrated implant encased in adequate cortical bone could successfully survive a severe sudden traumatic impact of substantial force.

CONCLUSIONS

Dental implants are protected in cortical bone from traumatic occlusion impact. It is due to collagen, the matrix of bone, which helps to prevent bone from fracture. In our case, the occlusion forces were strong enough to ceramic fracture on crown and loosen the abutment retaining screw. The impact force caused no apparent damage to the bone, the implant or its components except abutment screw loosening. The force delivered at the incisal edge of the crown (axial direction) probably induced a reverse torque to the implant and did not result in its failure or bone resorption.

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