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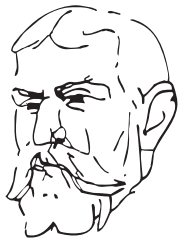

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CONTENS / SPIS TREŚCI

ORIGINAL ARTICLES / PRACE ORYGINALNE

ORIGINAL ARTICLE
PRACA ORYGINALNA

SKIN MICROBIAL LANDSCAPE AND IMMUNE-ENDOCRINE PARAMETERS IN PATIENTS WITH PSORIASIS BY USING NARROWBAND UVB PHOTOTHERAPY

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ABSTRACT

The aim: The study of skin microbial landscape and immune-endocrine parameters as well as improvement of treatment efficiency in patients with different clinical course of psoriasis by using narrowband UVB phototherapy.

Materials and methods: We examined 89 patients with psoriasis (51 men and 38 women) aged 18-60. The comparison group consisted of 43 psoriasis patients comparable by age, sex and clinical course with those from the main group (46 patients). Cytokine levels IL-4, IL-8, IL-10, TNFα, thyroid peroxidase (TPO) and thyroglobulin (Tg) autoantibodies and microbial flora of skin were determined in patients with psoriasis.

Results: The study finds that conventional therapy does not have sufficient corrective impact on immune-endocrine disorders and the use of narrow-band light therapy has shown that it has a focused corrective impact on cytokine production and modulating effect on the level of TPO and Tg autoantibodies and the state of skin automicroflora of lesions in patients suffering from psoriasis.

Conclusions: Using UVB (311 nm) therapy in patients with psoriasis allows improving efficiency of treatment and limiting clinical signs in the form of achieving remission and significant improvement in patients' health without any negative dynamic changes.

KEY WORDS: psoriasis, etiopathogenesis, skin microbial landscape, immune-endocrine disorders, phototherapy

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INTRODUCTION

Psoriasis is a systemic disease characterized by a complex of mutually conditioned pathogenetic links, i.e. immune, neuroendocrine, infectious etc. One of the main factors of morphological changes is accelerated epidermis proliferation, which occurs due to the reduction of cell cycle of epidermocytes [1,2].

An inherent pathogenetic mechanism of psoriatic disease is its mediated changes in the cytokine profile [3,4]. It should be noted that despite numerous studies of the interleukin cascade in psoriasis patients the results obtained and their interpretation are often quite controversial [5,6,7,8]. Therefore, we consider that it is necessary to focus on a more in-depth study of the relationship between individual cytokines, the association of their levels with the clinical psoriasis course and the state of other systems and organs in such patients. In such a perspective, based on the world and domestic scientific sources, according to the above subject, as well as based on our own clinical experience and studies of immunological parameters in psoriasis patients, we believe, the study of levels of L-4, IL-8, IL-10, TNFα, which have both antiinflammatory and pro-inflammatory activity, is the most expedient except for pathogenetic IL-17 and IL-23.

Endocrine disorders play an important role in the development of this dermatosis, in particular an increase in the level of thyroidism is observed, which is associated with the deterioration of environmental conditions, and gain in neurological symptoms in the population. Thyroid disorders associated with its oppressed and increased functional activity are characterized by the development of autoimmune reactions, the appearance of circulating thyroglobulin antibodies, microsomal and thyroid stimulating immunoglobulins, which in turn enhances the allergic component. In addition, it should be remembered that the metabolism of thyroid hormones occurs in the skin. Nevertheless, studies of autoantibodies to thyroperoxidase (TPO) and thyroglobulin (TG) in psoriasis patients with look promising [9].

It is known today that skin biocenosis condition may be a highly sensitive indicator of the sensitization fidelity. It is proved that a microorganism and its microbial flora while physiological conditions are in a state of dynamic equilibrium [10]. Therefore, in each part of the body surface microflora is characterized by a relative stability. Possible changes in a microorganism are reflected in disorders of the microbial landscape of all skin topographic areas. It should be noted that numerous studies of skin microbial flora in psoriasis patients are often very episodic and not always structured, so they require further and more detailed in-depth study [11].

Despite the large number of suggested methods and means of treatment of psoriasis patients their effectiveness often remains not too high [12]. At present, there are a number of questions concerning etiology, multifactorial development, polymorphism of clinical manifestations, etc., which, in turn, does not allow developing a unified therapeutic concept [13]. According to modern clinical guidelines for the treatment of psoriasis patients, the use of 311 nm narrowband UVB therapy is pathogenetically conditioned since it produces a selective effect on skin structures and demonstrates powerful antiinflammatory and immunomodulatory properties. It is proved that waves of this band are the most therapeutically reasonable as they allow providing a significant rate of remission and duration of positive treatment results preservation than the use of waves of other bands [14]. In addition, UVB (311nm) does not require the use of chemotherapy, in turn, it does not cause to their specific side effects and does not have a number of contraindications that occur when using PUVA-therapy [15].

Thus, the review of studies on immunopathogenesis of psoriasis disease, the state of skin microbiocenosis and therapeutic tactics illustrates an open nature of the problem. The available data are scattered and are often accumulated chaotically. The information content of certain indicators reflecting individual mechanisms of psoriasis development is not defined. Therefore, their further study undoubtedly expands the scope of understanding of dermatosis development mechanisms and is quite promising for subsequent devising of coordinated therapeutic intervention and control of the therapy efficacy in such patients.

THE AIM

The aim of our research is to increase the efficacy of treatment through studying changes in the skin microbial landscape and some immunological and endocrine parameters in patients with different clinical course of psoriasis against the background of the use of narrowband UVB therapy.

MATERIALS AND METHODS

We observed 89 psoriasis patients (51 men and 38 women) who were determined the level of IL-4, IL-8, IL-10, TNFα content in their blood serum by means of a test system ("Protein circuit") implemented in a "sandwich-method" of solid-phase immunoenzyme analysis using horseradish peroxidase as an enzyme indicator following the recommendations by the manufacturer. The results were registered per the activity of the bound peroxidase using STAT-FAX-303 PLUS device at 492 nm wave extension. The IL level is expressed in pg/ml.

All the observed by us patients were examined for skin microbial flora taken from focal lesions in three stages. At the first identification stage a cultural study was carried out. In order to identify and quantify the microorganismal exchange on the skin surface we prepared culture dishes with a nutrient medium consisting of 5% blood agar and Sabouraud agar. At the next stage, bacterioscopic examination of bacterial colonies plated from the skin as well as isolation of pure microbial cultures was performed. Then, the identification of the isolated cultures by their enzymatic properties on classical differential-diagnostic media was carried out with the following recalculation of the number of colonies grown on each CFU per 1 cm^2.

Autoantibody levels to TPO and TG were detected by a solid-phase sandwich enzyme immunoassay method using reagent kits AT-TPO-IFA and AT-TG-IFA in accordance with the established methodology and instructions. Data received were expressed in IU/ml.

All psoriasis patients under our observation were evaluated per the Psoriasis Area and Severity Index (PASI) and the Dermatological Quality of Life Index (DLQI). The criteria for inclusion of patients in the study were such as psoriasis vulgaris (PV) and PV in combination with psoriatic arthritis (PsA), age of patients from 18 to 60 years, written consent of a patient to participate in the study. Criteria for excluding patients from the study were as follows: current erythrodermic and pustular forms of psoriasis, contraindications to the use of phototherapy, comorbidities that may significantly affect outcomes of the study, and participation in any other study.

In order to clarify the degree of corrective effect of narrow-band phototherapy on clinical manifestations of dermatosis, immunomicrobiological disorders and quality of life of patients we carried out a comparative analysis of its efficacy and efficacy of traditional therapeutic means.

All patients were divided into two groups. The first (main) group was formed by 46 patients with psoriasis vulgaris, which was verified in 35 (76.1%) patients, and PV in combination with psoriatic arthritis, which was diagnosed in 11 (23.9%) patients who had contraindications for the use of cytostatic therapy. The second group (comparative) consisted of 43 psoriasis patients comparable by age, sex and clinical course with those from the main group.

The main group of patients underwent UVB therapy on Daavlin 3 Series PC 311-24 unit using TL-01 lamps generating radiation in 310-315 nm and with a peak emission at a length of 311 nm in combination with conventional treatment means according to the modern clinical guidelines and in the light of available PsA. The initial dose was 0.1-0.2 J/cm^2 and depended on the skin phototype, which was determined by testing. With each subsequent procedure it increased by 0.05-0.1 J/cm^2. If erythema was observed, the dose remained the same. Procedures were performed 3-4 times a week. The total number of procedures ranged from 12 to 18 and depended on the clinical course of psoriasis.

The comparative group was provided with traditional means and methods of therapy, i.e. hyposensitizing, antihistamine, non-steroidal anti-inflammatory medications, B vitamins, topical glucocorticosteroids, D3 vitamins, emollients, keratolytics under the modern clinical guidelines and in the light of available psoriatic arthritis. The treatment course lasted for 4 weeks.

The research results were statistically processed by using Microsoft Office Excel 2016 (Windows 10 Professional).

Table I. Changes in cytokine content within the comparison group psoriasis patients during their treatment course.

Indicators (M±m), pg/ml	Groups of examined patients			
	Patients with psoriasis vulgaris		Patients with psoriasis vulgaris and PsA	
	Before treatment	After treatment	Before treatment	After treatment
IL-4	67.31±3.02	65.12±4.82	74.38±2.65	71.37±3.90
IL-8	54.13±2.06	41.19±3.78	64.07±2.58	66.25±5.17
IL-10	36.51±1.85	33.87±2.94	37.13±1.52	25.94±1.82
TNFα	63.75±3.74	60.95±7.12	68.51±3.19	52.14±3.19

Table II. Changes in cytokine levels in the main group psoriasis patients during their treatment.

Indicators (M±m), pg/ml	Groups of examined patients			
	Patients with psoriasis vulgaris		Patients with psoriasis vulgaris and PsA	
	Before treatment	After treatment	Before treatment	After treatment
IL-4	67.31±3.02	48.19±3.24	74.38±2.65	56.49±4.01
IL-8	54.13±2.06	42.37±2.15	64.07±2.58	48.13±1.64
IL-10	34.51±1.85	15.98±3.39	37.13±1.52	23.08±1.43
TNFα	63.75±3.74	51.17±1.76	68.51±3.19	49.23±2.19

We determined the value of arithmetic mean value (M), the mean square deviation (G) and error in determining the arithmetic mean (m). The level of confidence of significance of differences (P) was determined using the Student's t-criterion. To determine the reliability of a relationship between two variation series, the correlation coefficient was calculated using such formula as $r = \sum H / \sum M$, where r is the correlation coefficient; $\sum H$ is the sum of products of deviations from the arithmetic mean of the first and second series; $\sum H$ is the maximum sum. A negative value indicated an inverse correlation, while a positive value indicated a direct correlation. The closer to the value 1 is value 2, the more likely a relationship between the indicators is.

RESULTS

During the examination of 89 psoriasis patients it was noticed that among factors initiating the development of a pathological process the most frequent were nervous and psychic ones, bacterial and viral infections in 43.3% and 24.5% of patients respectively. It should be noted that 32.2% of patients could not indicate the probable cause of dermatosis manifestation. Hereditary predisposition to psoriasis development is traced in 43.9% of patients. Associated pathology was observed in 25.7% of patients. Psoriasis relapses occurred mainly during autumn-winter period in 68.2% of patients, the spring-summer type was identified in 11.5% of patients, absence of seasonal influence – in 20.3%. Progressive course of psoriasis was established in 66.5% of patients, and in-patient – in 33.5%. The PASI index ranged from 18 to 65 points.

The result of examination of patients from the comparison group upon the application of recommended therapy testify only a slight correction of cytokines content (Table 1). A statistically valid confirmation of the reduction of IL-8 level was obtained only in patients with PV, i.e. up to 41.19±3.78 pg/ml (before treatment – 54.13±2.06 pg/ml; $p < 0.05$), IL-10 level in patients with psoriasis vulgaris in combination with PsA, i.e. up to 25.94±1.82 pg/ml (before treatment – 37.13±1.52 pg/ml; <0.05) and TNFα level in the same category of patients, i.e. up to 52.14±3.19 pg/ml (before treatment – 68.51±3.19 pg/ml; $p < 0.05$). However, the value of these parameters remained beyond the physiological oscillation line.

Probable suppression of autoantibodies to TG was registered in comparison group patients with psoriasis vulgaris and PsA (up to 130.16±9.83 IU/ml (before treatment – 156.48±7.12 IU/ml; $p<0.05$). It should be noted that this indicator also remained outside the amplitude of physiological fluctuations.

The degree of microbial skin contamination in psoriasis patients from the comparison group also decreased significantly. In patients with PV the number of S. aureus fell to 316.32±16.11 CFU/cm (before treatment – 415.81±14.25 CFU/cm, $p<0.05$). S. epidermidis – up to 57.29±4.08 CFU/cm (before treatment – 93.40±5.38 CFU/cm, $p<0.05$) S. saprophyticus – up to 20.17±1.96 CFU/cm (before treatment – 37.65±3.02 CFU/cm, $p<0.05$) Micrococcus spp. – up to 17.13±2.45 CFU/cm (before it was 25.19±2.16 CFU/cm, $p<0.05$) and Bacillus spp. – up to 61.42±2.17 CFU/cm (before treatment – 78.15±3.12 CFU/cm, $p<0.05$). However, as indicated by the data, the degree of microbial skin contamination in such patients remained outside the limits of physiological fluctuations. Only the number of other microorganisms did not exceed the control values of 8.04±0.34 CFU/cm (before treatment – 7.92±0.81 CFU/cm, $p> 0.05$). Microbial contamination in patients with psoriasis vulgaris in combination with psoriatic arthritis has changed likewise.

The quality of life of such patients improved. but this process shows no signs of possible justification. In particular. in patients with PV DLQI was 17.1±3.0 points

(before treatment – 18.2±2.4 points; p>0.05). and within the presence of PsA – 21.6±2.8 points (before treatment – 23.5±1.9 points. p>0.05).

The analysis of clinical efficacy of treatment of patients from the comparison group allowed stating the absence of "clinical remission" in all cases of observation, "significant improvement" – in 11 (26.1%) people, "improvement" – in 17 (39.1%), "without changes" – in 13 (30.4%), "deterioration" - in 2 (4.3%). Dermatosis relapses within 12 months after the end of therapy were registered in 30 (69.6%) patients.

It has been established that upon receiving their recommended treatment patients from the main group had a reliable decrease in cytokines activity (Table 2). In patients with psoriasis vulgaris of the disease IL-4 and IL-10 levels reached the limits of physiological fluctuations amounting to 48.19±3.24 pg/ml (before treatment – 67.31±3.02 pg/ml; p>0.05) and 15.98±3.39 pg/ml (before treatment – 34.51±1.85 pg/ml; p<0.05) respectively. Despite a significant decrease, the IL-8 and TNFα content remained outside the control values, amounting to 42.37±2.15 pg/ml (before treatment – 54.13±2.06 pg/ml; p<0.05) and 51.17±1.76 pg/ml (before treatment – 63.75±3.74 pg/ml; p<0.05), respectively. Against the presence of PsA, cytokine activity was also confidently suppressed though remained outside the physiological range. Thus, the IL-4 level reached 56.49±4.01 pg/ml (before treatment – 74.38±2.65 pg/ml; p<0.05) IL-8 – 48.13±1.64 pg/ml (before treatment – 64.07±2.58 pg/ml; p<0.05) IL-10 – 23.08±1.43 pg/ml (before treatment – 37.13±1.52 pg/ml; p<0.05) and TNFα – 49.23±2.19 pg/ml (before treatment – 68.51±3.19 pg/ml; p<0.05).

The study of autoantibody contents allowed stating the preservation of their physiological level to TPO in case of psoriasis vulgaris up to 14.72±0.97 IU/ml (before treatment – 15.07±1.54 IU/ml; p<0.05) and TG – up to 81.25 ± 4.12 IU/ml (before treatment – 86.75±5.80 IU/ml, p> 0,05). Against the presence of psoriatic arthritis, their levels are registered as decreased to physiological values. In particular, antibodies to TPO were up to 15.02 ± 0.89 IU/ml (before treatment – 25.73 ± 0.95 IU/ml; p> 0.05), and to TG – up to 85.62±6.24 IU/ml (before treatment – 156.48±7.12 IU/ml, p> 0.05).

After the treatment applied, the microbial contamination of focal skin eruptions was significantly suppressed in patients from the main group. The number of *Staphylococcus aureus* and *Staphylococcus epidermidis* in patients with PV decreased significantly, remaining however outside the physiological range and amounted up to 235.83±24.39 CFU/cm^2 (before treatment – 415.81±14.25 CFU/cm^2, p<0.05) and 42.38±1.95 CFU/cm^2 (before treatment – 93.40±5.38 CFU/cm^2, p<0.05) respectively. The levels of *Staphylococcus saprophyticus*, *Micrococcus spp*. and *Bacillus spp*. were reduced to the values of 14.29±2.54 CFU/cm^2 (before treatment – 37.65±3.02 CFU/cm^2, p>0.05); 9.16±2.14 CFU/cm^2 (before treatment – 25.19±2.16 CFU/cm^2, p>0.05) and 20.08±1.89 CFU/cm^2 (before treatment – 78.15±3.12 CFU/cm^2, p>0.05) respectively. The number of other microorganisms remained in the range of physiological fluctuations, i.e. 9.21±1.64 CFU/cm^2 (before treatment – 7.92±0.81 CFU/cm^2, p>0.05). Similarly, the concentration of microorganisms changed in the case of psoriasis vulgaris and PsA, making it 250.13±15.19 CFU/cm^2 (before treatment – 525.12±31.78 CFU/cm^2) 94.11±4.62 CFU/cm^2 (before treatment – 142.36±15.83 CFU/cm^2, p<0.05); 13.02±1.76 CFU/cm^2 (before treatment – 53.94±2.95 CFU/cm^2, p<0.05); 8.16±0.62 CFU/cm^2 (before treatment – 35.18±1.74 CFU/cm^2, p<0.05); 19.29± 2.14CFU/cm^2 (before treatment – 95.87±4.13 CFU/cm^2, p<0.05) and 8.85±1.12 CFU/cm^2 (before treatment – 8.19±0.50 CFU/cm^2, p>0.05) respectively.

In patients with PV the DLQI was 14.0±0.8 points (before treatment – 18.2±2.4 points; p<0.05); in the presence of PsA – 16.1±1.2 (before treatment – 23.5±1.9 points; p<0.05.

The performed analysis of clinical expediency of the use of narrowband phototherapy allowed confirming the achievement of "clinical remission" in 32 (69.6%) of patients, "significant improvement" in 9 (19.6%), and "improvement" in 5 (10.8%). There were no cases of no positive changes or deterioration of the condition. In 12 (26.1%) of patients, psoriasis relapses occurred during 1 year after the end of treatment, but their severity was significantly lower than in patients from the comparison group.

DISCUSSION

Analysing the obtained results, it can be stated that in patients from the main group there is a reliable suppression of cell activity in respect of cytokine production, the degree of which depends on the clinical course of dermatosis and significantly exceeds similar values in patients from the comparison group. It is proved that the recommended method of phototherapy, which was used to treat psoriasis patients from the main group produces a modulating effect on the levels of autoantibodies to TPO and TG, and significantly suppresses microbial contamination. Accordingly, the rate of DLQI and PASI indices decreases, and these were especially significant in patients from the main group as compared to the insignificant fluctuations in patients from the comparison group.

Thus, the presented data testify that an integrated use of narrowband UVB therapy in psoriasis patients is better in respect of the efficacy as compared to the monotherapy by traditional means of treatment due to its systemic anti-inflammatory, immunomodulatory and antimicrobial effect. Such a multidirectional effect, in turn, allows identifying this methodology as a limiting the pathological process through the clinical and pathogenetic influence.

CONCLUSIONS

1. It is shown that traditional therapeutic means such as monotherapy produce insufficient corrective effect on immuno-microbiological disorders and clinical manifestations in psoriasis patients.
2. It has been established that narrowband phototherapy in combination with traditional means shows a more

accentuated corrective effect on the cytokines production, modulating effect on the content of autoantibodies to TPO and TG and the condition of automicroflora of focal skin eruptions in psoriasis patients.

3. It has been proved that the combination of UVB-therapy (311 nm) and traditional methods of treatment of patients with various clinical courses of psoriasis is effective, safe, convenient enough to use and allows obtaining in 70% of cases a complete clinical and predictable remission.
4. It is stated that the use of this combined method allows increasing the efficacy of treatment and limit clinical manifestations of psoriasis in the form of remission, significant improvement of patients' condition and quality of life in the absence of negative dynamic changes.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

ASSOCIATION ANALYSIS BETWEEN *HOTAIR* RS1899663 SINGLE NUCLEOTIDE POLYMORPHISM AND CLEAR CELL RENAL CELL CARCINOMA DEVELOPMENT IN UKRAINIAN POPULATION

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ABSTRACT

The aim: to study the association between rs1899663-polymorphic variant of *HOTAIR* gene and clear cell renal cell carcinoma (CCRCC) development in Ukrainian population.
Materials and methods: whole venous blood from 101 Ukrainians with CCRCC (42 females and 59 males) and 100 control subjects (34 females and 66 males) were enrolled in the study. DNA extraction was performed using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, USA). Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was used for *HOTAIR* rs1899663 genotyping. The Statistical Package for Social Science software (SPSS, version 17.0, Chicago, IL, USA) was used for all calculations.
Results: It was found the lack of association between *HOTAIR* rs1899663 single nucleotide polymorphism and CCRCC emergence as well as tumor metastasis property in dominant, recessive, over-dominant and additive crude models of inheritance, as well after the adjustment for age, sex, smoking and excessive alcohol consumption ($P > 0.05$).
Conclusions: No association was found between *HOTAIR* rs1899663-polymorphic variant and CCRCC development in Ukrainian population. Further studies with extended samples are required to validate these results.

KEY WORDS: clear cell renal cell carcinoma, long non-coding RNA, HOTAIR, gene polymorphism

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INTRODUCTION

It has always been believed that mutations are the basis of cancer development. But since the discovery of epigenetics, it has become clear that the combination of genetic and epigenetic changes can lead to cancer [1]. Epigenetics studies changes in gene expression that are not related to variation in DNA sequence [2]. There are some epigenetic modifications described in the literature: DNA methylation, histone acetylation or deacetylation and histone methylation [3].

The main part of transcripts is represented by non-coding RNAs (ncRNAs), which divided into two major groups: short and long. Long ncRNAs (lncRNA) have more than 200 nucleotides and are mainly transcribed by RNA polymerase II. They are involved in gene regulation, cell differentiation and development, inactivation of X chromosome etc. One of the representatives of lncRNA is Hox transcript antisense intergenic RNA (HOTAIR), which was discovered by Rinn et al [4].

The *HOTAIR* is located at 12 chromosome inside the HoxC locus, particular, between HoxC11 and HoxC12 and includes 12 649 nucleotides. The lncRNA HOTAIR turns on 2 158 nucleotides. HOTAIR can act due to its interaction with different complexes, for example Polycomb repressive complex 2 (PRC2) and Lysine-specific histone demethylase 1 (LSD1), effect on the expression of miRNAs, such as miR-122, miR-17-5p, miR-206, regulate epithelial mesenchymal transition (EMT) etc. [5-8]. Due to PRC2 and LSD1, methylation and demethylation of the corresponding genes are carried out, which leads to a decrease in their expression. For example, these complexes inhibit genes such as tumor and metastasis suppressor genes (*RKIP*, *PSP94*, *Kruppel-like factor 2*, *RUNX3*, *SLIT2*, *DAB2IP* etc.) [5, 9-14].

Moreover, it has been found that the overexpression of *HOTAIR* moderately increases the rate of growth of the primary tumor and promotes the metastasis of cancer. It was observed an overexpression of *HOTAIR* in renal cell carcinoma cells in comparison with normal renal tissue [15].

Clear cell renal cell carcinoma (CCRCC) is the most common subtype of renal cell carcinoma (RCC), which is characterized by accumulation of lipid and glycogen in the cytoplasm of tumor cells that leads to the predominance of clear cell histology [16]. It is the most common and malignant cancer, because of its high metastatic potential, rates of invasiveness and mortality. It accounts for about 65%–75% of all RCCs [17, 18, 19]. There are a lot of risk factors for CCRCC, including genetic, epigenetic, and modifiable factors. The main modifiable factors are age, sex, excessive alcohol consumption and cigarette smoking. [20].

A single-nucleotide polymorphism (SNP) is the most common type of genetic variation which means the variation in a single nucleotide that has a specific position in the genome. Genes containing SNPs can produce several allelic forms of ncRNAs, which can differ in their effect on gene regulation, chromatin modification and so on [21]. Today, 3 828 single-nucleotide polymorphisms of the *HOTAIR* in

Table I. Clinical characteristics of the patients with CCRCC and control.

Parameter	CCRCC (n = 101)	Control (n = 100)	P
Age, years ± SD	55.31 ± 10.41	77.38 ± 8.49	< 0.001
Sex, female/male	42/59	34/66	0.268
Smokers, n (%)	49 (48.51)	27 (27)	0.002

CCRCC – clear cell renal cell carcinoma; n – number of cases; P – indicator of statistical significance. Categorical variables were compared by χ^2-test, quantitative variables - by t-test.

Table II. Clinical characteristics of the patients with and without CCRCC metastasis.

Parameter	With metastasis (n = 29)	Without metastasis (n = 72)	P
Age, years ± SD	58.03±11.54	54.21±9.78	0.095
Sex, female/male	11/18	31/41	0.636
Smokers, n (%)	14 (48.3)	35 (48.6)	0.976
Drinkers, n (%)	18 (62.1)	46 (63.9)	0.864

CCRCC – clear cell renal cell carcinoma; n – number of cases; P – indicator of statistical significance. Categorical variables were compared by χ^2-test, quantitative variables - by *t*-test.

humans (NCBI) have been studied. The essence of *HOTAIR* rs1899663-polymorphism is the replacement of guanine by thymine at the 12 chromosome at 53967210th position. It is located in the second intron site in 4903 position (according to NC_000012.12 and NR_003716.3) [22].

Therefore, it was decided to check the association between *HOTAIR* rs1899663-polymorphism and the development of CCRCC in Ukrainian population.

THE AIM

The aim was to study the association between rs1899663-polymorphic variant of *HOTAIR* and clear cell renal cell carcinoma (CCRCC) development in Ukrainian population.

MATERIALS AND METHODS

STUDY POPULATION

It was used the whole venous blood of 101 Ukrainians with CCRCC (42 females and 59 males; mean age [±SD] 55.31±10.41) and 100 subjects (34 females and 66 males; mean age 77.38 ± 8.49) as a control (Table I). It was found 29 subjects with metastasis (11 females and 18 males; mean age 58.03±11.54) among oncological patients (Table II).

Each cancer patient was diagnosed from March 2001 to May 2016 with further observation in Sumy Regional Clinical Oncology Dispensary. All oncological patients underwent radical tumor removal with further histological examination. All cancer patients had clinical stage II (TNM Classification of Malignant Tumors). Final morphological diagnosis of CCRCC was estimated according to the European Association of Urology Guidelines. It is worth paying attention to the fact that the mean age of the control group is significantly higher than that in cancer group. Thereby, the risk of cancer development in the control subjects decreases with age that improves the reliability of the control group. The study protocol complied with the Declaration of Helsinki and was approved by the Ethic Committee of the medical Institute of Sumy State University (№3/05.12.11). All individuals gave voluntary informed written consent.

GENOTYPING

DNA was isolated from venous blood of 201 subjects using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, USA). Polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) was used for genotyping *HOTAIR* rs1899663 SNP. It were used the 2 mM $MgSO_4$, 0.2 mM dNTPs (Thermo Fisher Scientific, USA), 5 µL 5 × PCR buffer, 1 U Taq DNA polymerase (Thermo Fisher Scientific, USA), and 75–100 ng DNA for the reaction mixture for PCR (total volume 25 µL). The nucleotide structure of the primers, PCR stages and PCR amplicon size are shown in the Table III. PCR was conducted to in Thermocycler GeneAmp PCR System 2700 (Thermo Fisher Scientific, USA).

The primers were chosen to limit the 401 bp DNA sequence, which contains the rs1899663-polymorphic site. However, due to the presence of a constitutional restriction site, the amplicon was split by the BseG1 endonuclease into two parts of 76 and 325 bp. An additional BseG1 restriction site was formed due to the transversion of G → T in 4093 (rs1899663-polymorphism) position of the *HOTAIR* (NC_000012.12). At the same time, along with the formation of a fragment of 76 bp, a fragment of 325 bp was cut into two additional ones – 63 and 262 bp. Instead, only two fragments – 76 bp and 325 bp were formed in the presence of the G-allele in the amplicon. Horizontal electrophoresis (10 V/cm) in 2.5% agarose gel with the

Table III. PCR conditions for *HOTAIR* rs1899663-polymorphism.

Primers	PCR stages			Amplicon size
Forward: 5'TGAAAGCCAGGATCATTTAACA3'	Denaturation	Hybridization	Elongation	401 bp
Reverse: 5'GGGCTCATGGAGACATTTTAAG3'	94°C – 45 s	59°C – 45 s	72°C – 45 s	

Note: bp: base pairs

Table IV. Distribution of alleles and genotypes for the *HOTAIR* rs1899663-polymorphism in case and control groups.

	CCRCC (n =101)		Control (n = 100)		P_{HWE}	P
	n	%	n	%		
Genotypes						
GG	40	39.6	35	35	-	0.207
GT	53	52.5	49	49		
TT	8	7.9	16	16		
Alleles						
G	181	64.2	119	59.5	0.862	0.189
T	101	35.8	81	40.5		

CCRCC – clear cell renal cell carcinoma; n – number of cases; PHWE – the rate of deviation of allele frequencies from the Hardy-Weinberg equilibrium; P – indicator of statistical significance.

Table V. Analysis of the association between the *HOTAIR* rs1899663-polymorphism and the development of CCRCC.

Model	P_c	OR_c (95% CI)	P_a	OR_a (95% CI)
Dominant	0.5	0.821 (0.463-1.456)	0.631	0.864 (0.475-1.571)
Recessive	0.083	0.452 (0.184-1.109)	0.140	0.497 (0.196-1.258)
Over-dominant	0.622	1.149 (0.661-1.999)	0.614	1.161 (0.650-2.074)
Additive[1]	0.092	0.438 (0.167-1.145)	0.163	0.492 (0.182-1.331)
	0.857	0.946 (0.521-1.720)	0.957	0.983 (0.526-1.835)

CCRCC – clear cell renal cell carcinoma; Pc: crude P value; ORc: crude odds ratio; CI: confidence interval; P_a: P value adjusted for age, sex and smoking; OR_a: adjusted odds ratio. [1] Upper row in the additive model of inheritance – comparison between TT and GG genotypes; lower row – between GT and GG genotypes.

addition of a bromide ethidium solution (10 mg / ml) was used to separate the restriction products. Discrimination of *HOTAIR* rs1899663-polymorphism genotypes was performed by the transilluminator ("Biocom", Russia).

STATISTICAL ANALYSIS

The Online Calculator of Hardy-Weinberg equilibrium (https://wpcalc.com/en/equilibrium-hardy-weinberg) was used to determine the distribution of alleles in groups of comparison and Hardy-Weinberg equilibrium (HWE) testing. All computations were made in the Statistical Package for Social Science software (SPSS, version 17.0, Chicago, IL, USA). Chi square (χ^2) test (comparing the frequency of alleles and genotypes and other variables) and two-tailed Student's t-test (comparison of averages between two groups) were used in the study. Shapiro-Wilk test confirmed the normal distribution. It was used the logistic regression to estimate the odds ratio (OR) and 95 % confidence interval (CI) in the framework of recessive, dominant, over-dominant and additive inheritance models. Multivariable logistic regression was used for smoking, sex, age, and excessive alcohol consumption adjustment. All statistical tests were based on a two-tailed probability; a value of $P < 0.05$ was accepted as significant.

Table VI. Distribution of alleles and genotypes for the *HOTAIR* rs1899663-polymorphism among patients with and without CCRCC metastasis.

	With metastasis (n = 29)		Without metastasis (n = 72)		P
	n	%	n	%	
Genotypes					
GG	12	41.4	28	38.9	0.790
GT	14	48.3	39	54.2	
TT	3	10.3	5	6.9	
Alleles					
G	38	65.5	95	66.0	0.951
T	20	34.5	49	34.0	

CCRCC – clear cell renal cell carcinoma; n – number of cases; P – indicator of statistical significance.

RESULTS AND DISCUSSION

The clinical characteristics of the study groups are shown in Table I. The cancer group was included 101 individuals with CCRCC with 55.31 ± 10.41 as an average age. The control group was consisted of 100 subjects with an average age of 77.38 ± 8.49, which was much higher. Thus, between cancer and control groups the significant difference was found in average age ($P < 0.001$) as well, as in smokers ($P = 0.002$). At the same time, there were no significant differences in sex distribution between these two groups ($P = 0.268$).

The distribution of alleles and genotypes in case and control groups is presented in Table IV. Alleles distribution

Table VII. Analysis of the association between the *HOTAIR* rs1899663-polymorphism and the development of metastasis of CCRCC.

Model	P_c	OR_c (95% CI)	P_a	OR_a (95% CI)
Dominant	0.817	0.902 (0.375-2.169)	0.880	0.933 (0.382-2.279)
Recessive	0.569	1.546 (0.345-6.938)	0.608	1.489 (0.325-6.829)
Over-dominant	0.592	0.790 (0.333-1.873)	0.666	0.824 (0.342-1.984)
Additive[1]	0.677	1.400 (0.287-6.818)	0.694	1.381 (0.277-6.872)
	0.703	0.838 (0.337-2.083)	0.773	0.872 (0.345-2.203)

CCRCC – clear cell renal cell carcinoma; Pc: crude P value; ORc: crude odds ratio; CI: confidence interval; P_a: P value adjusted for age, sex, smoking and excessive alcohol consumption; OR^a: adjusted odds ratio. 1Upper row in the additive model of inheritance – comparison between TT and GG genotypes; lower row – between GT and GG genotypes.

matched with HWE expectation (P_{HWE} = 0.862). Both the distribution of genotypes and alleles had no significant differences (P_g = 0.207; P_a = 0.189).

The results of analysis of the association between the *HOTAIR* rs1899663-polymorphism and the development of CCRCC are summarized in Table V. There was no association in all crude models of inheritance as well as after the adjustment for covariates ($P > 0.05$).

Moreover, 29 subjects with metastasis among oncological patients were found, but there was no significant difference in all parameters: age (P = 0.095), sex (P = 0.636), smokers (P = 0.976), drinkers (P = 0.864) (Table II). Also, there was no association in distribution of alleles and genotypes among patients with and without CCRCC metastasis (P_g = 0.790; P_a = 0.951) (Table VI).

The results of analysis of the association between the *HOTAIR* rs1899663-polymorphism and the development of metastasis of CCRCC are represented in Table VII. There was no significant difference in all crude regression models. Also, there was no association after the adjustment for sex, age, smoking and excessive alcohol consumption ($P > 0.05$).

In this study we have checked the association between *HOTAIR* rs1899663-polymorphism and the development of CCRCC in Ukrainian population. The *HOTAIR* is located at 12 chromosome inside the HoxC locus and includes 12 649 nucleotides. It was found by John L. Rinn et al. (2007) as a regulator of Chromatin Silencing [4]. They also described in detail the mechanism of HOTAIR´s action through PRC2.

Since that time, many scientists studied the association between *HOTAIR´s* overexpression and development of different types of cancer. In 2010 Miao-Chih Tsai et al. showed that a 3' domain of HOTAIR binds the LSD1/CoREST/REST complex [16]. Zhi-Yuan Xu et al. (2013) have found that inhibition of *HOTAIR* reverses EMT in gastric cancer [17]. Xiao-Song Ge et al. (2013) showed that HOTAIR inhibits Wnt inhibitory factor 1 expression and activates Wnt pathway. The Wnt/b-catenin signaling pathway plays an important role in migration and cell proliferation and cancer progression [18]. Liu XH et al. (2014) showed that HOTAIR can act as a competing endogenous RNA. Through miR-331-3p it can regulate *HER2* expression in gastric cancer [19]. Mohammadreza Hajjari and Abbas Salavaty (2015) showed the role of *HOTAIR´s* overexpression, based on information from various articles. Studies have shown a positive link between the *HOTAIR´s* overexpression and cancer progression and metastasis. Further, they gathered together all the factors that may affect on *HOTAIR´s* expression. For example, Ago2 complex can suppress the function of *HOTAIR* unlike osteopontin, which causes it´s overexpression [5].

This lncRNA has a lot of different polymorphisms, so we have chosen one of them. The essence of *HOTAIR* rs1899663-polymorphism is the replacement of guanine by thymine at the 12 chromosome at 53967210th position [22]. It is located in the intronic region of *HOTAIR* [23]. There are three major *HOTAIR* transcript variants that have an appropriate SNP localization: variant 1- 325+59G>T (NR_047517.1), variant 2 – 266+59G>T (NR_003716.3), variant 3 – 299-833G>T (NR_047518.1).

During the study we discovered the following T-allele distribution for Ukrainian population: 0.358 for cancer group and 0.405 for control group. There were no significant differences in distribution of alleles in case and control groups. The T-allele distribution in non-metastasis and metastasis groups was 0.34 and 0.345 respectively. Also, there were no significant differences in distribution of alleles in these two groups.

The 1000 Genomes Project showed the minor allele frequency in 5 populations: East Asian (EAS) – 0.205, South Asian (SAS) – 0.31, European (EUR) – 0.268, African (AFR) – 0.179 and American (AMR) – 0.36. The most similar results for Ukrainian control group were obtained only for American (0.36). So, there are ethnic differences in the rs1899663 allele´s distribution [22].

The number of works devoted to this polymorphism was significantly increased over the past few years. Xu Yang et al. (2018) have found a significant association between rs1899663 C>A (P = 0.29) polymorphism and increased neuroblastoma risk [24]. At the same time, most authors such as Shun-Long Weng et al. (2018), Min-Che Tung et al. (2019), Xu T et al. (2019) etc. have not found a significant association between rs1899663 polymorphism and cancer development [25-27].

At this study we investigated only the association between the *HOTAIR* rs1899663-polymorphism and CCRCC development. It is of interest to study the *HOTAIR* expression levels depending on the allelic variant.

CONCLUSIONS

In this work it was studied the involvement of rs1899663-polymorphism in the development of CCRCC. The alleles and genotypes distribution of rs1899663-polymorphism for Ukrainian population was investigated. It was found no significant differences between *HOTAIR* rs1899663-polymorphism and CCRCC as in most works of other authors. It can be concluded that there are more significant predictors.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

KSZTAŁTOWANIE SIĘ POZIOMU WIEDZY PIELĘGNIAREK NA TEMAT SEPSY W ZALEŻNOŚCI OD SPECYFIKI ODDZIAŁU SZPITALNEGO I STAŻU PRACY W ZAWODZIE

THE LEVEL OF KNOWLEDGE OF SEPSIS AND SEPTIC SHOCK AMONG NURSES DEPENDS ON PROFESSIONAL EXPERIENCE AND TYPE OF HOSPITAL WARD THEY WORK

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STRESZCZENIE

Cel pracy: Ocena kształtowania się poziomu wiedzy pielęgniarek na temat sepsy w zależności od specyfiki oddziału szpitalnego i stażu pracy w zawodzie.

Materiał i metody: Materiał badań stanowiła losowo wybrana grupa 100 pielęgniarek i pielęgniarzy pracujących na trzech różnych oddziałach: Szpitalny Oddział Ratunkowy (SOR), Oddział Intensywnej Terapii (OIT) oraz oddział chirurgii. Narzędziem badawczym był autorski kwestionariusz ankiety. Badania były przeprowadzane w maju 2019 roku.

Wyniki: Wiedza ogólna pielęgniarek i pielęgniarzy na temat sepsy jest dość dobra. Większość ankietowanych poprawnie odpowiadało między innymi na pytanie o definicję sepsy i wstrząsu septycznego, przyczyny sepsy i istotne dla niej parametry w badaniach laboratoryjnych. Niestety bardziej szczegółowe pytania, jak np. kryteria, które pomagają przy rozpoznaniu sepsy czy możliwość całkowitego jej wyleczenia sprawiały więcej trudności. Można było zauważyć także brak posiadania aktualnej wiedzy.

Wnioski: Powinno się kłaść większy nacisk na dostępność aktualnych informacji na temat sepsy i weryfikowanie wiedzy personelu pielęgniarskiego. Należy zadbać o zwiększenie świadomości wagi posiadania aktualnej wiedzy w kontekście jak najwyższej jakości i skuteczności sprawowanej opieki nad pacjentem. Pielęgniarki i pielęgniarze pracujący na Szpitalnym Oddziale Ratunkowym i na Oddziale Intensywnej Terapii podsiadają większy zasób wiedzy na badany temat od personelu Oddziału Chirurgii.

SŁOWA KLUCZOWE: sepsa, wstrząs septyczny, pielęgniarki, SOR, OIT, chirurgia

ABSTRACT

The aim: Assessment of the level of knowledge of nurses of sepsis depending of type of hospital word and years of working experience.

Materials and methods: The audit was carried out in the group of 100 randomly selected nurses working in three different hospital wards: Hospital Emergency Ward, Intensive Care Unit and Surgery Unit. Study dedicated authors survey was developed as audits research tool. The study was conducted in May 2019.

Results: The general knowledge of nurses of sepsis is quite good. Most of the respondents answered correctly to questions about definition of sepsis and septic shock, causes of sepsis and relevant laboratory parameters. Unfortunately, more detailed questions, about diagnostic criteria or chances of complete cure, caused more difficulties. Some responders lack up-to-date knowledge about sepsis and septic shock.

Conclusion: More emphasis should be placed on the availability of up-to-date information on sepsis and verification of the knowledge of nurses working in hospital are should be taken to raise awareness and stress importance of up-to-date knowledge in order to provide the highest quality and utmost effectiveness of patient care. Nurses working in the Hospital Emergency Ward and Intensive Care Unit have more knowledge about the subject of our study than the staff in the Surgery Unit.

KEY WORDS: sepsis, septic shock, nurses, Hospital Emergency Ward, Intensive Care Unit, Surgery Unit

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WSTĘP

Systematycznie odnotowuje się wzrastającą liczbę przypadków sepsy i wstrząsu septycznego. W państwach rozwiniętych liczba notowanych przypadków narasta około 9 % w skali roku i szacuje się ją na około 50–95 przypadków na populację liczącą 100 tysięcy osób [1].

Z badań, które zostały przeprowadzone w Polsce w latach 2012–2013 wynika, że sepsa to powód leczenia około 25%

Tabela. 1. Struktura wieku respondentów.

	Poniżej 28 lat	Od 28 do 33 lat	Od 33 do 39 lat	Od 39 do 44 lat	Od 44 do 50 lat	Od 50 do 55 lat	Powyżej 55 lat
Liczba osób	47	11	11	11	13	6	1
Odsetek [%]	47,0	11,0	11,0	11,0	13,0	6,0	1,0

Tabela 2. Odpowiedzi respondentów na pytanie 1: Czym jest sepsa?

Odpowiedzi	Liczność	Odsetek [%]
zagrażająca życiu dysfunkcja narządowa spowodowana zaburzoną regulacją odpowiedzi ustroju na zakażenie	91	91,00
nie zagrażająca życiu dysfunkcja narządowa spowodowana zaburzoną regulacją odpowiedzi ustroju na zakażenie	2	2,00
dysfunkcja narządowa spowodowana tylko i wyłącznie przez wirusy	6	6,00
nie zagrażająca życiu dysfunkcja spowodowana przez zakażenie wirusami i bakteriami Gram-ujemnymi	1	1,00
Suma końcowa	100	100,00

pacjentów na Oddziale Intensywnej Terapii. Szacuje się, że sepsa na OIT w Polsce występuje w 65 przypadkach na 100 tys. populacji [1].

Warto zaznaczyć, że śmiertelność spowodowana wstrząsem septycznym jest bardzo wysoka – około 40–80% na Oddziale Intensywnej Terapii. Sepsa i wstrząs septyczny mogą wystąpić w każdej grupie wiekowej, mogą dotyczyć każdego – dlatego tak ważne jest zdobywanie i aktualizowanie wiedzy na ten temat [1, 2].

SEPSA I WSTRZĄS SEPTYCZNY

Sepsa jest to zagrażająca życiu dysfunkcja narządowa spowodowana niewłaściwą reakcją organizmu na zakażenie [2].

Jej najczęstszą przyczyną są zakażenia bakteryjne dróg oddechowych, zakażenia wewnątrzbrzuszne, zakażenia ran operacyjnych, krwi, ośrodkowego układu nerwowego i układu moczowego [3, 4].

SEPSIS – 3

Zgodnie z najnowszymi wytycznymi kryteria, dzięki którym rozpoznajemy dysfunkcję narządową w sepsie to skala SOFA i qSOFA. Zastąpiły one popularny do tej pory SIRS (*systemic inflammatory respone syndrome* – zespól uogólnionej reakcji zapalnej) [5].

Według Sepsis-3 wyróżnią się tylko sepsę i wstrząs septyczny. Podział na sepsę, ciężką sepsę i wstrząs septyczny, jak miało to miejsce przy użyciu SIRS – jest już nieaktualny. Wstrząs septyczny to sepsa, w której szczególnie ciężkie zaburzenia komórkowe i metaboliczne oraz zaburzenia ze strony układu krążenia niosą za sobą dużo większe ryzyko zgonu niż sama sepsa [2].

Punktacja SOFA używana jest na Oddziałach Intensywnej Terapii, natomiast poza OIT stosuje się kryteria quickSOFA. W skali SOFA – skali niewydolności narządów związanych z sepsą przyznaje się punkty od 0 do 4 za każdy z wymienionych aspektów: wskaźnik oksygenacji, liczba płytek krwi, stężenie bilirubiny w surowicy, średnie ciśnienie tętnicze oraz stosowanie leków wazopresyjnych, ocena świadomości w skali Glasgow oraz stężenie kreatyniny w surowicy i/lub wielkość diurezy. Nagłe zwiększenie wyniku w skali SOFA o co najmniej 2 punkty wskazuje na sepsę, jeśli występuje lub podejrzewa się zakażenie [6].

Według qSOFA ryzyko wystąpienia sepsy jest wtedy, gdy u pacjenta stwierdza się dwa z trzech: zmiany stanu świadomości; wartości skurczowego ciśnienia krwi nie wyższe niż 100 mmHg; przyspieszenia częstości oddechowej ≥ 22 na minutę.

W 2017 roku zostały opublikowane przez *Surviving Sepsis Campaing* nowe międzynarodowe wytyczne dotyczące postępowania w sepsie i we wstrząsie septycznym. Pakiet zadań według *Surviving Sepsis Campaign* powinien być przeprowadzony w ciągu 1 godziny od zaobserwowania objawów wskazujących na sepsę.

Pakiet ten zawiera takie czynności, jak:

- oznaczenie stężenia mleczanów we krwi,
- pobranie krwi na posiewy przed zastosowaniem antybiotyków,
- zastosowanie antybiotyków o szerokim spektrum działania,
- rozpoczęcie szybkiego przetaczania 30 ml/kg roztworu krystaloidów, jeśli występuje hipotensja lub stężenie mleczanów we krwi wynosi ≥4 mmol/l,
- zastosowanie leków obkurczających naczynia w razie hipotensji niereagującej na wstępną intensywną płynoterapię, aby utrzymać średnie ciśnienie tętnicze ≥65 mm Hg [7, 8]

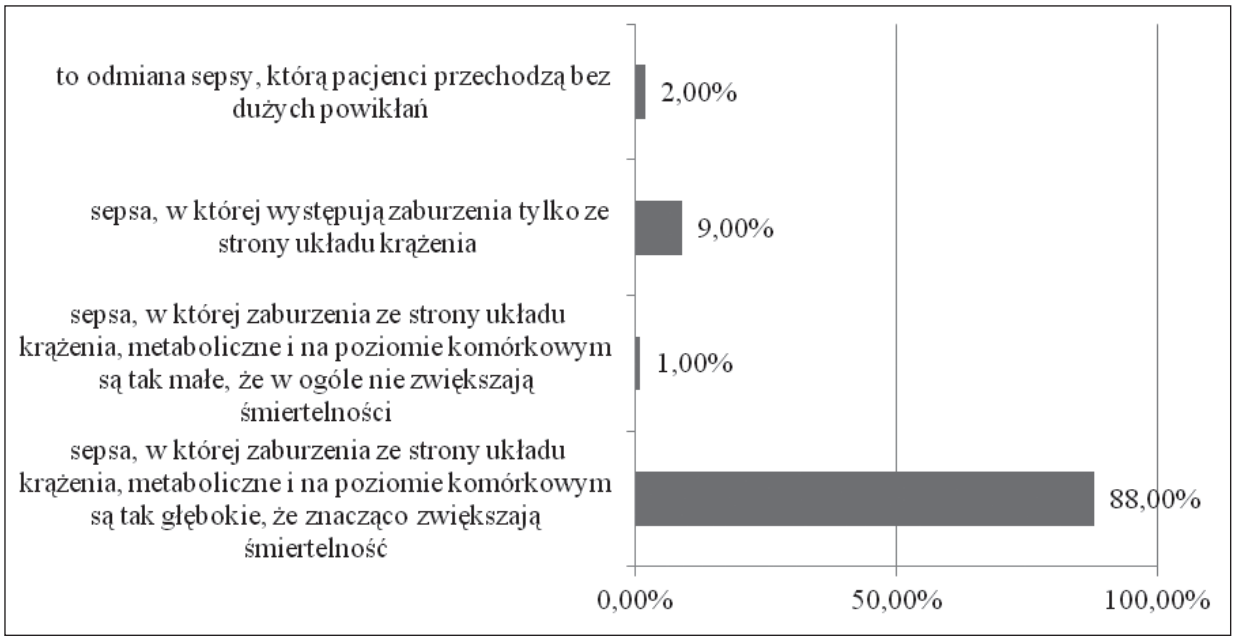
CEL

Celem badań była ocena kształtowania się poziomu wiedzy pielęgniarek na temat sepsy w zależności od specyfiki oddziału szpitalnego i stażu pracy w zawodzie.

MATERIAŁ I METODY

Materiał badań stanowiła losowo wybrana grupa 100 pielęgniarek i pielęgniarzy pracujących na trzech różnych oddziałach: Szpitalny Oddział Ratunkowy (SOR), Oddział Intensywnej Terapii (OIT) oraz Oddział Chirurgii.

Narzędziem badawczym w tym badaniu był autorski kwestionariusz ankiety. Ankieta składała się z 20 pytań: 19



Ryc. 1. Odpowiedzi respondentów na pytanie 2: Wstrząs septyczny to….

Tabela 3. Odpowiedzi respondentów na pytanie: Czy sepsa i wstrząs septyczny może wystąpić w każdej grupie wiekowej?

Odpowiedzi	Liczność	Odsetek [%]
Tak	95	95,00
Nie	5	5,00
Suma końcowa	100	100,00

Tabela 4. Odpowiedzi respondentów na pytanie: Za pomocą jakich kryteriów rozpoznaje się sepsę?

Odpowiedzi	Liczność	Odsetek [%]
Skali SOFA i qSOFA	54	54,00
SIRS	43	43,00
BLS	3	3,00
Suma końcowa	100	100,00

zamkniętych i 1 otwartego – krótkiej odpowiedzi. Udział w badaniu był dobrowolny, a ankiety anonimowe. Badania były przeprowadzane w maju 2019 roku. W czasie przeprowadzania badania rozdane zostało 146 ankiet, lecz do badania zakwalifikowano tylko 100 z nich.

Do analizy statystycznej wykorzystany został program Statistica PL. Weryfikację hipotez statystycznych przeprowadzono przy użyciu testu chi-kwadrat . Istotność różnic pomiędzy zmiennymi badano za pomocą testu t-Studenta. Za poziom istotności przyjęto 0,05.

WYNIKI

Wśród ankietowanych były 92 kobiety (92% ankietowanych) oraz 8 mężczyzn (8% ankietowanych).

Tabela 5. Odpowiedź na pytanie: Skala niewydolności narządów związana z sepsą to…

Odpowiedzi	Liczność	Odsetek [%]
SOFA	44	44,00
SIRS	48	48,00
MNA	6	6,00
NORTON	2	2,00
Suma końcowa	100	100,00

Tabela 6. Odpowiedź respondentów na pytanie: Co uważa się za obowiązkowy marker biochemiczny sepsy?

Odpowiedzi	Liczność	Odsetek [%]
Mleczany	59	59,00
GGTP	26	26,00
pCO2	14	14,00
Potas	1	1,00
Suma końcowa	100	100,00

Wiek respondentów przedstawia Tabela 1.

Badanych było 100 osób. Średni wiek ankietowanych został oszacowany na poziomie 33,96 roku.

W grupie ankietowanych najwięcej było osób z wykształceniem licencjata i stanowiły one 59% ogółu. Magistrów było 30%, a osób z wykształceniem średnim 11%.

Staż pracy w zawodzie wśród respondentów był zróżnicowany. 38% ankietowanych miało staż pracy w zawodzie pielęgniarki / pielęgniarza powyżej 10 lat, 34% do 2 lat oraz 22% od 3 do 6 lat.

Respondenci równomiernie byli rozłożeni pomiędzy 3 oddziały, czyli 33% pracowało na Szpitalnym Oddziale

Tabela 7. Odpowiedź respondentów na pytanie: Według qSOFA ryzyko wystąpienia sepsy jest wtedy, gdy u pacjenta stwierdza się dwa z trzech wymienionych objawów.

Odpowiedzi	Liczność	Odsetek [%]
zmiany stanu świadomości; wartości skurczowego ciśnienia krwi nie wyższe niż 100 mmHg; przyspieszenia częstości oddechowej wyższa lub równe 22 na minutę	67	67,00
zmiany stanu świadomości, wartości skurczowego ciśnienia krwi nie wyższe niż 150 mmHg; przyspieszenia częstości oddechowej wyższe lub równe 22 na minutę	16	16,00
wartości skurczowego ciśnienia krwi nie wyższe niż 150 mmHg; przyspieszenia częstości oddechowej wyższe lub równe 10 na minutę	7	7,00
brak zmian stanu świadomości, wartości skurczowego ciśnienia krwi nie wyższe niż 100 mmHg; przyspieszenia częstości oddechowej wyższe lub równe 22 na minutę	10	10,00
Suma końcowa	100	100,00

Tabela 8. Odpowiedź na pitanie: Pakiet zadań według Surviving Sepsis Campaign powinien być przeprowadzony w ciągu _____ od zaobserwowania objawów wskazujących na sepię

Odpowiedzi	Liczność	Odsetek [%]
1 godziny	42	42,00
2 godzin	31	31,00
30 minut	21	21,00
3 godzin	6	6,00
Suma końcowa	100	100,00

Tabela 9. Odpowiedź na pytanie: Nagłe zwiększenie wyniku w skali SOFA o co najmniej _____ wskazuje na sepsę, jeśli występuje lub podejrzewa się zakażenie.

Odpowiedzi	Liczność	Odsetek [%]
2	37	37,00
5	15	15,00
3	39	39,00
1	9	9,00
Suma końcowa	100	100,00

Tabela 10. Odpowiedź respondentów na pytanie: Czy jest możliwe całkowite wyleczenie sepsy?

Odpowiedzi	Liczność	Odsetek [%]
Tak ale jej następstwem mogą być trwałe powikłania.	64	64,00
Tak	27	27,00
Nie, tylko częściowe	8	8,00
Nie wiem	1	1,00
Suma końcowa	100	100,00

Ratunkowym, tyle samo na Oddziale Intensywnej Terapii i 34% na chirurgii.

Pytanie 1. Na pierwsze pytanie dotyczące tego czym jest sepsa, większa część ankietowanych odpowiedziała prawidłowo, co widoczne jest w Tabeli 2.

Pytanie 2. Wstrząs septyczny to ….Wstrząs septyczny prawidłowo definiuje aż 88% respondentów, a pozostałe 12 % odpowiada błędnie (Ryc. 1).

Pytanie 3. Sepsa i wstrząs septyczny to stany zagrożenia życia.

96% pielęgniarek i pielęgniarzy wie, że sepsa i wstrząs septyczny to stany zagrożenia życia.

Pytanie 4. Czy sepsa i wstrząs septyczny może wystąpić w każdej grupie wiekowej?

W tym pytaniu 95% ankietowanych odpowiedziało prawidłowo (Tab. 3).

Pytanie 5. dotyczyło przyczyn sepsy.

Głównymi przyczynami sepsy wskazywanymi przez 87% respondentów były zakażenia bakteryjne dróg oddechowych, zakażenia wewnątrzbrzuszne, zakażenia krwi, zakażenia ran operacyjnych, zakażenia układu moczowego, zakażenia ośrodkowego układu nerwowego i była to odpowiedź prawidłowa.

Pytanie 6. Za pomocą jakich kryteriów rozpoznaje się sepsę? (Tab. 4).

Z tabeli wynika, że 54% pielęgniarek i pielęgniarzy zna prawidłową odpowiedź.

Pytanie 7. Czy sepsa i wstrząs septyczny oznaczają to samo?

Sepsa i wstrząs septyczny nie oznaczają tego samego i takiego zdania jest 85% respondentów. 13 % błędnie odpowiada, że oznaczają to samo, a 2% twierdzi, że nie wie.

Pytanie 8.

Skala niewydolności narządów związana z sepsą to…. (Tab. 5)

Niestety aż 48% respondentów odpowiedziało na to pytanie błędnie. Tylko 44% znało prawidłową odpowiedź.

Pytanie 9. Jaki jest istotny parametr w badaniach laboratoryjnych, który umożliwia rozpoznanie sepsy po wystąpieniu objawów SIRS? (Ryc. 2)

Na to pytanie prawidłowo odpowiedziało 84 % ankietowanych.

Pytanie 10. Jakie jest leczenie przyczynowe w sepsie?

96% respondentów podaje prawidłowo, iż leczenie przyczynowe w sepsie to antybiotykoterapia i usunięcie ogniska zakażenia.

Pytanie 11. Co uważa się za obowiązkowy marker biochemiczny sepsy? (Tab. 6)

Prawidłowy obowiązkowy marker biochemiczny wskazało 59% ankietowanych.

Tabela 11. Wyniki testu t: z dwiema próbami zakładający równe wariancje (badanie 1).

	Pielęgniarki pracujące w SOR i OIT	Pielęgniarki pracujące na chirurgii
Średnia	22,57576	10,00
Wariancja	16,49417	10,84848
Obserwacje	66	34
Wariancja sumaryczna	14,59307	
Różnica średnich wg hipotezy	0	
df	98	
t Stat	15,59453	
P(T≤t) jednostronny	1,35E-28	
Test T jednostronny	1,660551	
P(T≤t) dwustronny	2,69E-28	
Test t dwustronny	1,984467	

Tabela 12. Wyniki testu t: z dwiema próbami zakładający równe wariancje (badanie 2

	Pielęgniarki będące bezpośrednio po studiach	Pielęgniarki ze stażem pracy powyżej 10 lat
Średnia	11,23529	10,158
Wariancja	4,306595	12,73115
Obserwacje	34	38
Wariancja sumaryczna	8,759575	
Różnica średnich wg hipotezy	0	
df	70	
t Stat	1,542056	
P(T≤t) jednostronny	0,063784	
Test T jednostronny	1,666914	
P(T≤t) dwustronny	0,127569	
Test t dwustronny	1,994437	

Pytanie 12. Według qSOFA ryzyko wystąpienia sepsy jest wtedy, gdy u pacjenta stwierdza się dwa z trzech wymienionych objawów (Tab. 7).

Na pytanie o ryzyko wystąpienia sepsy 67% ankietowanych odpowiedziało prawidłowo, a pozostałe 33% niestety nie.

Pytanie 13. Pakiet zadań według Surviving Sepsis Campaign powinien być przeprowadzony w ciągu _____ od zaobserwowania objawów wskazujących na sepię (Tab. 8).

Prawidłowy czas wskazało tylko 42% ankietowanych.

Pytanie 14. Nagłe zwiększenie wyniku w skali SOFA o co najmniej _____ wskazuje na sepsę, jeśli występuje lub podejrzewa się zakażenie (Tab. 9).

Niestety tylko 37% respondentów odpowiedziało prawidłowo na to pytanie.

Pytanie 15. Czy jest możliwe całkowite wyleczenie sepsy? (Tab. 10)

64% respondentów zgadza się, że możliwe jest całkowite wyleczenie sepsy, ale jej następstwem mogą być trwałe powikłania.

BADANIE 1

Pielęgniarki pracujące w SOR i OIT posiadają większą wiedzę na temat sepsy od pielęgniarek pracujących na oddziale chirurgii. Aby móc oceniać wiedzę ankietowanych przyznawano punkty za wskazania prawidłowych odpowiedzi i w ten sposób z wszystkich pytań otrzymano wynik sumaryczny dla każdej osoby. Odpowiedź prawidłowa była dawał respondentowi 1 pkt, zaś nieprawidłowa zero. Dzięki zbieraniu danych o punktacji możliwe było porównanie wiedzy różnych grup, czyli np. z podziałem na odziały, w których wykonują pracę. W celu weryfikacji powyższej hipotezy wykonano test t-Student różnicy średnich (Tab. 11):

H0: hipoteza zerowa mówi, że średnia punktacja za wiedzę w obu grupach pielęgniarek jest statystycznie równa

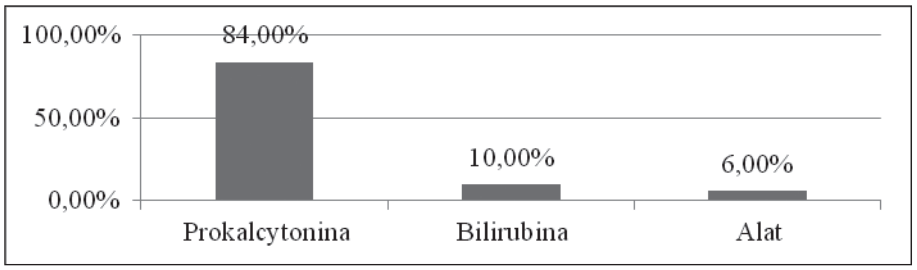
H1: hipoteza alternatywna mówi, że średnia punktacja za wiedzę w obu grupach pielęgniarek jest statystycznie różna

Z uwagi na obliczoną wartość p=2,69E-28, czyli bliską zera i mniejszą od przyjętego poziomu istotności 0,05 odrzucamy hipotezę zerową i stwierdzamy, iż średnie są istotnie różne.

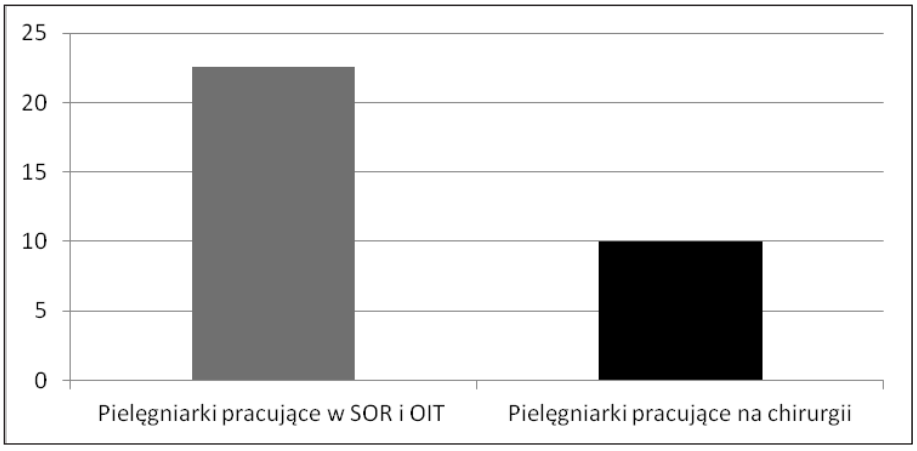
Wyraźnie średnia punktacja za wiedzę u pielęgniarek pracujących w SOR i OIT (22,6 pkt) jest większa od wiedzy pielęgniarek z chirurgii (10 pkt) (Ryc. 3).

BADANIE 2

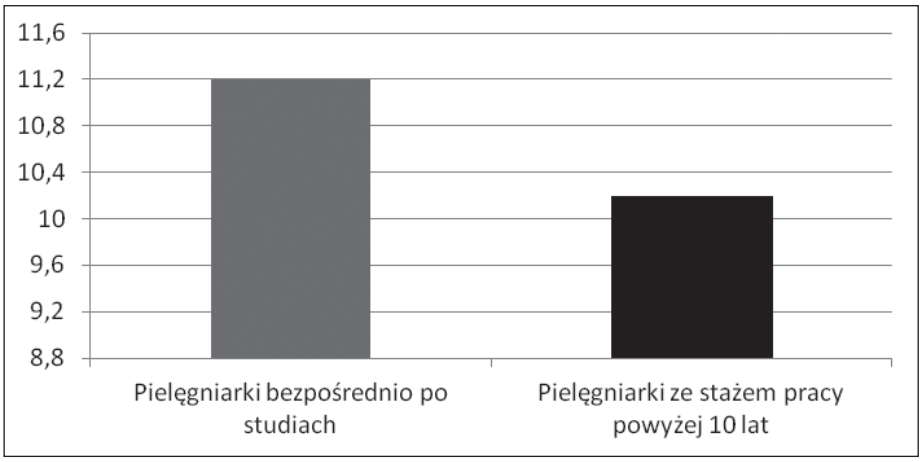
Zakładam, że pielęgniarki będące bezpośrednio po studiach, posiadają większy zasób wiedzy na temat sepsy od pielęgniarek ze stażem pracy powyżej 10 lat.



Ryc. 2. Odpowiedź respondentów na pytanie: Jaki jest istotny parametr w badaniach laboratoryjnych, który umożliwia rozpoznanie sepsy po wystąpieniu objawów SIRS?



Ryc. 3. Ocena wyników testu wiedzy z uwzględnieniem miejsca pracy.



Ryc. 4. Ocena wyników testu wiedzy z uwzględnieniem stażu pracy.

W celu weryfikacji powyższej hipotezy wykonano test t-Student różnicy średnich:

H0: hipoteza zerowa mówi, że średnia punktacja za wiedzę w obu grupach pielęgniarek jest statystycznie równa

H1: hipoteza alternatywna mówi, że średnia punktacja za wiedzę w obu grupach pielęgniarek jest statystycznie różna

Z uwagi na obliczoną wartość p=0,12757 większą od przyjętego poziomu istotności 0,05 brak podstaw do odrzucenia hipotezy zerowej i stwierdzam, iż średnie są statystycznie równe. Pomimo iż wynik średniej punktacji za wiedzę pielęgniarek bezpośrednio po studiach jest wyższy (11,2 pkt) od pielęgniarek ze stażem powyżej10 lat (10,2 pkt) to jednak różnica ta nie jest istotna statystycznie. Należy uznać, iż wyniki z wiedzy obu grup pielęgniarek są statystycznie równe (Tab. 12, Ryc. 4).

DYSKUSJA

Po przeprowadzonym badaniu wiedzę pielęgniarek i pielęgniarzy na temat sepsy można określić jako dość dobrą, lecz wymagającą pogłębiania i aktualizacji. Ze względu na to, że na przełomie ostatnich kilku lat zaszły duże zmiany w definicji sepsy i w kryteriach jej diagnostyki, ważna jest aktualizacja wiedzy przez personel pielęgniarski. Mimo wielu zmian w definicji, jest jeden wspólny mianownik – konieczność wczesnej identyfikacji choroby i szybkie wdrożenie leczenia. Twierdzi się, że szybkość postawienia diagnozy ma największy wpływ na dobre wyniki leczenia sepsy i wstrząsu septycznego [9].

Dzięki badaniom przeprowadzonym w Królestwie Arabii Saudyjskiej w latach 2011–2013 wiemy, że każda godzina, która mija między wystąpieniem pierwszych objawów hipotensji a podaniem antybiotyków, zmniejsza szanse na przeżycie pacjenta o 7,6% [10].

Natomiast w badaniach przeprowadzonych na terenie Tajwanu w latach 2007–2010 porównywano skuteczność leczenia sepsy u pacjenta na OIT w zależności od długości stażu pracy lekarza. Badanie to wykazało, że staż pracy lekarza na OIT obniża śmiertelność w przypadku sepsy

u osób dorosłych [11]. Dzięki temu wnioskować można, że odpowiednie doświadczenie i wiedza szeroko pojętego personelu medycznego skutkuje lepszym rokowaniem pacjentów z sepsą lub wstrząsem septycznym. Pielęgniarka i pielęgniarz to osoby, które spędzają najwięcej czasu z pacjentem w czasie hospitalizacji, ale i przy rutynowych kontaktach w przychodni i innych miejscach spotkań i edukacji. Dzięki zaktualizowanej wiedzy personelu pielęgniarskiego, możliwe jest natychmiastowe zaobserwowanie zmian w samopoczuciu pacjenta oraz w jego funkcjach i parametrach życiowych.

Szczególnie pomocna w opiece nad potencjalnym pacjentem septycznym dla pielęgniarek i pielęgniarzy jest skala qSOFA, ponieważ nie wymaga ona wykonywania badań laboratoryjnych, a opiera się na pomiarze liczby oddechów na minutę, pomiarze ciśnienia tętniczego krwi oraz ocenie stanu psychicznego pacjenta [12].

Należy pamiętać też, że Ustawa o Zawodach Pielęgniarki i Położnej, nakłada na personel pielęgniarski konieczność ciągłego aktualizowania swojej wiedzy i umiejętności [13].

Pielęgniarki i pielęgniarze nie tylko mają możliwość prawidłowego zinterpretowania objawów sepsy za pomocą skali qSOFA, ale także do nich należy edukacja społeczeństwa, między innymi na temat: symptomów sepsy, zapobieganiu infekcjom i zakażeniom oraz ich rozprzestrzenianiu się.

Badania prowadzone w Norwegii udowodniły, że wczesne rozpoznanie sepsy przez pielęgniarki u hospitalizowanych pacjentów zwiększyło ich przeżywalność w okresie 30 dni [14]. Z tego powodu wnioskować można o istotności znajomości pakietu zadań *Surviving Sepsis Campaign* przez personel pielęgniarski.

W marcu 2019 roku w wywiadzie dla everethnews.pl prof. Andrzej Kübler, specjalista anestezjologii i intensywnej terapii oraz Prezes Stowarzyszenia „Pokonać Sepsę" powiedział, że w Polsce wiedza dotycząca sepsy, jej rozpoznawania i realnego zagrożenia w polskich placówkach wciąż jest mała [15]. Zwrócił uwagę na to, że ważne jest interdyscyplinarne spojrzenie na temat sepsy oraz aktualizowanie i poszerzenie wiedzy wśród personelu medycznego. Twierdzi także, że sepsa jest takim samym zagrożeniem jak inne choroby społeczne, np. choroby układu krążenia czy nowotwory. Zaznaczył, że w obecnych czasach nie można rozwijać żadnej dziedziny medycyny, bez interdyscyplinarnego dialogu. Dzięki wyżej wspomnianemu dialogowi oraz aktualnej wiedzy członków zespołu jest mniejsza szansa na pominięcie istotnych faktów dotyczących stanu zdrowia pacjenta, tym samym zwiększając jego szanse na przeżycie i zmniejszając koszty i czas pobytu w szpitalu [15].

WNIOSKI

1. Badanie wykazało, że na faktyczny poziom wiedzy pielęgniarek i pielęgniarzy na temat sepsy nie wpływa staż pracy i czas, który upłynął od ukończenia studiów.
2. Oddział, na jakim pracowali respondenci ma istotny wpływ na ich zasób wiedzy na temat sepsy. Pielęgniarki pracujące w SOR i OIT posiadają większą wiedzę na temat sepsy od pielęgniarek pracujących na oddziale chirurgii.
3. Ogólna wiedza ankietowanych w powyższym temacie jest na poziomie dość dobrym. Wiedza zaawansowana i wiedza z koniecznością aktualizowania wypada znacznie gorzej.
4. Należy położyć nacisk na podniesienie świadomości personelu pielęgniarskiego w zakresie konieczności aktualizowania na bieżąco posiadanej wiedzy, zgodnie z aktualnymi danymi.
5. Od aktualnej wiedzy pielęgniarek i pielęgniarzy, którzy spędzają najwięcej czasu z pacjentem podczas hospitalizacji zależy zdrowie i życie człowieka. W przypadku sepsy istotna jest pierwsza godzina od momentu oceny stanu pacjenta – zgodnie z wytycznymi *Surviving Sepsis Campaign* z 2016 roku, dlatego ważne jest wczesne rozpoznanie objawów w oparciu o odpowiednie kryteria i skale.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

SNPS AND TRANSCRIPTIONAL ACTIVITY OF GENES OF INNATE AND ADAPTIVE IMMUNITY AT THE MATERNAL-FETAL INTERFACE IN WOMAN WITH PRETERM LABOUR, ASSOCIATED WITH PRETERM PREMATURE RUPTURE OF MEMBRANES

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ABSTACT

The aim is to identify mRNA expression of innate (TLR2 and TLR4) and adaptive (IL1 β, IL17A, FoxP3, Tbet, Roryt) immunity in maternal-fetal interface and evaluate the contribution of SNP genes of IL1β (rs1143627), TNFα (rs1800629), IL4 (rs2243250), IL10 (rs1800896, rs1800872) and RLN2 (rs4742076, rs3758239) to PTB, associated with PPROM in 26-34 weeks of gestation.

Materials and methods: We had done open cohort randomized research during period 2016-2018 years. The case group consisted of 50 women with PPROM in preterm pregnancy, 26-34 weeks of gestation. For the control group we collected samples from 50 women without previous history of PTB. To determine the level of mRNA target genes we used thermocycler CFX96™Real-Time PCR Detection Systems ("Bio-Rad Laboratories, Inc.", USA) and set of reagents Maxima SYBR Green / ROX qPCR MasterMix (2x) (Thermo Scientific, USA).

Results: In the population of the Zaporizhzhia region, there is no reliable clinical association between the IL1β and TNFα genes and a high risk of PTB. We obtained high reliable data on SNP genes RLN2 rs4742076 and rs3758239 in Zaporizhzhia women. The distribution of the rs2243250 gene polymorphism alleles of the IL4 gene of the main study group – TT homozygotes were determined in 2 (4%) cases, CT heterozygotes were found in 11 (22%), CC homozygotes in 37 (74%) cases. In the study of polymorphism rs1800872 of the IL10 gene, the main group of homozygous TT studies was identified in 7 (14%) cases, TG heterozygotes were found in 18 (36%), GG homozygotes in 25 (50%) cases. The range of all obtained values of the relative normalized expression of TLR2 gene in the placenta of 0.79-163.44 (median – 31.06), in the fetal membranes – 1.1-126.06 (median – 10.22). The placement of all obtained values compared to mRNA expression of the TLR4 gene was lower than the TLR2 in the placenta, which was 0.39-43.85 (median – 7.74) and higher in the fetal membranes – 0.18-216.01 (median – 40.04). We observed an 8.33-fold decreased expression in FoxP3 in decidua, especially in 31-32 weeks of PPROM manifestation (27.03-fold). In amniotic membranes a similar trend of reduction of FoxP3 expression was found, overall level decreased in 2.33 times, especially in 31-32 weeks of PPROM manifestation (10.64-fold).

Conclusions: Among Zaporizhzhia population, combination of IL4 (rs2243250), IL10 (rs1800896 and rs1800872), RLN2 (rs4742076 and rs3758239) supports the role for functional polymorphisms in immunoregulatory genes in the susceptibility to PTL, associated with PPROM. Marked increased transcriptional activity of components of innate (TLR2, TLR4), adaptive (Th1, Th17) immune system and conversely decreased expression of Treg (FoxP3) in the maternal-fetal interface are involved in immune pathways of PTB and contribute in the fetal inflammatory response syndrome.

KEY WORDS: single nucleotide polymorphism, transcriptional activity, preterm birth, preterm premature rupture of membranes

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INRODUCTION

Today, in Ukraine, the number of normal births is an average of 32.6% of their total. The frequency of registration of the prematurity in different regions of the country ranges from 3 to 12%. Spontaneous preterm birth (PTB) and preterm premature rupture of membranes (PPROM) are major contributors to neonatal mortality and serious neonatal morbidity worldwide [1, 2, 3].

It has been well-established that infection of the amniotic cavity can cause PTB via the activation of inflammatory processes resulting the onset of labour, rupture of membranes and dilatation oh the cervix [4]. This pathological inflammatory process can be caused by microorganisms invading the amniotic cavity (i.e. intra-amniotic infection) or danger signals/alarmins released during cellular stress or death (i.e. sterile intra-amniotic inflammation) [5]. In both scenarios, cytokines, such as interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor alpha (TNF-α) among others, play a central role in the pathophysiology of PTB [6]. Of all of these cytokines, IL-1β is a central mediator in the pathological process of PTB since it can stimulate the expression and release of other labor mediators, such as prostaglandins [7].

The epidemiological and clinical data regarding the proportions and relative incidence of intrauterine inflammation and infection in preterm deliveries are quite discordant

across different studies [8, 9]. Different patient populations exhibit different rates of vaginal dysbiosis, intrauterine infection and degrees of inflammatory responses [10, 11, 12].

Since inflammation is often invoked as an etiologic factor in spontaneous preterm birth, the question1 of whether spontaneous preterm birth has a genetic predisposition in the case of pathologic inflammation has been of long-standing interest to investigators. In recent years a great attention also has been paid to associative search of polymorphic markers with different diseases and as a result creation the genetic platform for personalized medicine. Most of the single nucleotide polymorphism (SNP) genes of the cytokines are found in the regulatory regions of the gene and directly affect their transcriptional activity and the concentration of the cytokine in the blood [13]. However, many findings are controversial what demonstrates the importance of standardized methods and reproductive techniques as well as strictly performed evaluation adjusted for potential confounding factors. Additionally, great part of inconsistencies found in the literature can be due to differences in genetic background and environmental exposures, parameters that vary greatly among distinct populations.

It is very important to identify changes in mRNA expression of immune cells at local level, to investigate candidate gene association with susceptibility to PTB induced PPROM and to integrate these results with our and other previous findings [14, 15, 16]. Through this mRNA analysis and integration with candidate gene sequencing data from the same individuals, we obtain a better understanding of the transcriptional regulation that occurs in the context of PTB.

THE AIM

To identify mRNA expression of innate (TLR2 and TLR4) and adaptive (IL1 β, IL17A, FoxP3, Tbet, Roryt) immunity in maternal-fetal interface and evaluate the contribution of SNP genes of IL1β (rs1143627), TNFα (rs1800629), IL4 (rs2243250), IL10 (rs1800896, rs1800872) and RLN2 (rs4742076, rs3758239) to PTB, associated with PPROM in 26-34 weeks of gestation.

MATERIALS AND METHODS

We had done open cohort randomized research during period 2016-2018 years. The case group consisted of 50 women with PPROM in preterm pregnancy, 26-34 weeks of gestation. For the control group we collected samples from 50 women without previous history of PTB. Control patients delivered normal infants at term without labor via elective cesarean section. Patients with diabetes mellitus, severe cardio-vascular morbidity, multiple gestations, fetal demise in utero or fetal anomalies were excluded.

Placental decidua and amniotic membranes tissues were obtained from patients (n=30) with PTB, associated with PPROM and control group (n=30). We are limited in our ability to perform extensive characterization, stratification/clustering or any predictive analyses of these mRNAs due to inadequate statistical power resulting from a small sample size. The study was approved by the Molecular-Genetic Research Division of the Medical and Laboratory Center of Zaporizhzhia State Medical University, and all patients who participated provided written informed consent. The tissues were collected at Regional perinatal center Zaporizhzhia city. The objects for molecular genetic studies with using of the real-time reverse transcription polymerase chain reaction (RT-PCR) techniques. Total RNA was procured from samples by use of "NucleoZOL" (Macherey-Nagel, Germany). For reverse transcription and obtaining cDNA, we used RevertAid First Strand cDNA Synthesis Kit ("ThermoScientific", USA). To determine the level of mRNA target genes we used thermocycler CFX96™Real-Time PCR Detection Systems ("Bio-Rad Laboratories, Inc.", USA) and set of reagents Maxima SYBR Green / ROX qPCR MasterMix (2x) (Thermo Scientific, USA). Specific primer pairs (5'-3') for analysis of target and reference genes were selected by the software PrimerBlast (www.ncbi.nlm.nih.gov/tools/primer-blast) and produced by ThermoScientific (USA). Normalized relative quantity of cDNA target genes was determined by the method $_{\Delta\Delta}$Ct. Statistical data analysis of PCR were conducted using available software CFX Manager ™ (Bio-Rad, USA).

We performed SNP sequencing in whole blood from women undergoing PTB induced PPROM with active contractions (26-34 weeks of gestation, n=50) matched for gestational age to healthy pregnant non-labouring controls (>37 weeks' gestation, n = 50) who later delivered at term.

The genotyping using TaqMan tests was done on amplifier CFX96™Real-Time PCR Detection Systems («Bio-Rad Laboratories, Inc.», USA). Polymerase chain reaction (PCR) for TaqMan genotyping was performed according to the instructions Applied Biosystems, USA. Studied SNPs genes were selected based on existing evidence in the literature for a role in the pathogenesis of the studied conditions for comparison with Ukrainian population.

Statistical data processing was carried out using the software package «Statistica 6.0» (StatSoftInc, No. AXXR712D-833214FAN5). The comparison of qualitative indices was carried out using the χ^2 criterion with Yates correction and Fischer's exact criterion (F). In order to evaluate the contribution of gene polymorphism to the probability of development of PPROM in preterm pregnancy, odds ratios (OR) were calculated with 95% confidence interval (CI). The differences were considered to be significant at $p < 0,05$.

RESULTS AND DISCUSSION

The data that we received in our work were compared with the results of other scientists. In addition, the results of studies of the same gene polymorphisms are determined by the design of the study are controversial in populations, and the expression of these mutations depends on a combination of culturological, socioeconomic and semantic factors, which are determined by the style of life in general and the style of habitation, in particular.

Table 1. Association between polymorphic locks of cytokine genes and high risk of PPROM and PTL in Zaporizhzhia region

SNPs	Allel / Genotype	Public location	OR	95 % CI	χ^2	p-value
IL1β *rs1143627*	Allele G Genotype GA Allele A	112836810	1,13 1,54 0,89	0,65-1,98 0,68-3,49 0,51-1,55	0,18	0,67
TNFα *rs1800629*	Allele A Genotype AG Allele G	31575254	0,8 0,67 1,25	0,42-1,54 0,28-1,62 0,65-2,39	0,44	0,51
IL4 *rs2243250*	Allele C Genotype CT Allele T	132673462	3,94 4,42 0,25	2,0-7,76 1,15-16,97 0,13-0,5	16,77	4×10^{-5} *
IL10 *rs1800896*	Allele C Genotype CT Allele TT	206773552	3,24 0,26 0,8	1,07-11 0,05-0,93 0,32-0,84	6,1	0,05 *
IL10 *rs1800872*	Allele T Genotype TG Allele G	206773062	0,33 0,2 24,0	0,18-0,58 0,08-0,47 5,25-109,65	14,7	0,0001 *
RLN2 *rs4742076*	Allele C Allele T	5309831	0,03 33,0	0,00-0,23 4,37-249,1	25,46	5×10^{-7} *
RLN2 *rs3758239*	Allele A Allele G	5306824	12,57 0,08	3,68-42,98 0,02-0,27	23,86	1×10^{-6} *

Note. *– reliability of the difference, $p<0.05$.

In connection with the above, there was a need for the study of frequent mutations of genes IL4 (rs2243250), IL10 (rs1800896 and rs1800872), IL1β (rs1143627), TNFα (rs1800629) and RLN2 (rs4742076 and rs3758239) in Zaporizhzhia population to determine their role in the pathogenesis of PPROM and PTL in order to identify patterns and mechanisms for the formation of PPROM. In Ukraine, the study of such a combination of genes in the pathology of software at the start of this study was not conducted.

Based on the genotyping results of rs1800896 gene polymorphism IL-10 gene TT homozygotes were detected in 37 (74%) cases of the main study group, CT heterozygotes in 2 (4%) and CC homozygotes in 11 (22%) cases, consequently. In the study of polymorphism rs1800872 of the IL10 gene, the main group of homozygous TT studies was identified in 7 (14%) cases, TG heterozygotes were found in 18 (36%), GG homozygotes in 25 (50%) cases. The distribution of the rs2243250 gene polymorphism alleles of the IL4 gene of the main study group – TT homozygotes were determined in 2 (4%) cases, CT heterozygotes were found in 11 (22%), CC homozygotes in 37 (74%) cases.

IL10 is an anti-inflammatory cytokine and attenuates the inflammatory response through effects on pro-inflammatory cytokines and reduces the function of host immune cells, such as neutrophils and macrophages. We proved association of IL4 rs2243250 with high risk or PPROM in Zaporizhzhia region, as in the study performed by Heinzmann A. in German population [17, 18].

In the study of polymorphism rs1143627 of the IL1β gene, the main group of homozygous AA studies was identified in 17 (34%) cases, GA heterozygotes were found in 21 (42%), GG homozygotes in 12 (24%) cases. The distribution of the rs1800629 gene polymorphism alleles of the TNFα gene of the main study group – AA homozygotes were determined in 5 (10%) cases, GA heterozygotes were found in 12 (24%), CC homozygotes in 33 (66%) cases. In the population of the Zaporizhzhia region, there is no reliable clinical association between the IL1β and TNFα genes and a high risk of PTB.

We have got statistically significant differences of rs4742076 polymorphisms (TT, CT, and CC) of the RLN2 gene in the study groups. We also detected statistically significant differences in all alleles of the rs3758239 polymorphism (AA, GG and AG) of the gene RLN2, respectively $p< 0.05$, indicating the reliability of the received prognostic markers (Table 1). The received data of frequency of alleles/genotypes distribution in case and control group is set on the figure 1 and 2.

A recent study by Vogel I. with homogeneous Danish population improved that women who are homozygous for specific SNP in the promotor region of RLN2 have a genetic susceptibility for PTL [19, 20]. In a study performed by Frederico G. Rocha et al. the contribution of SNP rs4742076 and rs 3758239 in the RLN2 promoter in Filipino population was shown [21, 22]. We obtained high reliable data on SNP genes RLN2 rs4742076 and rs3758239 in Zaporizhzhia women.

Summarizing the data of repertoire of SNP genes involved in our study, we have got significant difference in all markers, except IL1β (rs1143627), TNFα (rs1800629). The presence of these alleles may disrupt the balance between pro- and anti-inflammatory cytokines, modify the inflammatory response, increasing the risk for PPROM and PTB. Being a global problem preterm birth warrants global solutions. Recent genomic approaches are beginning to reveal the information for understanding the causes of PTL. To fully appreciate and

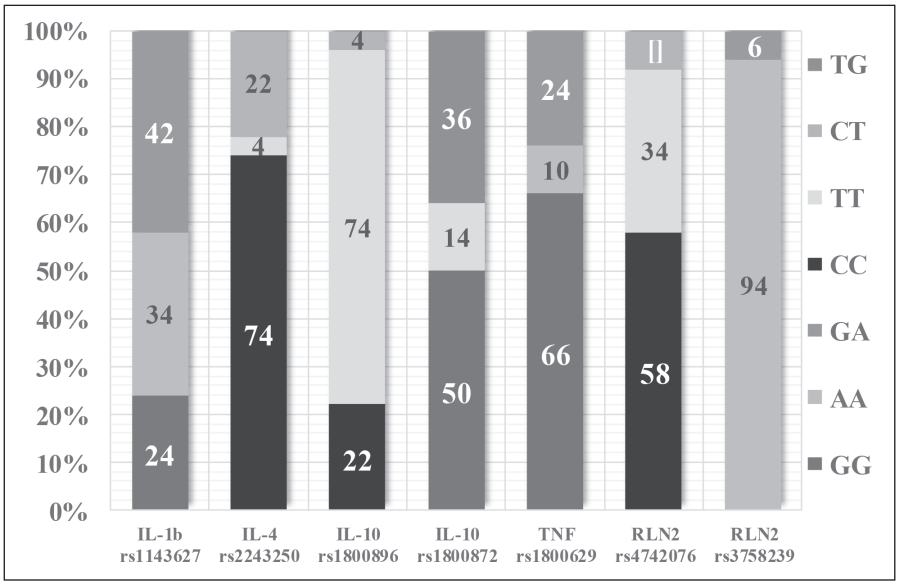

Fig 1. Frequency of genotypes distribution in women with PTB, associated with PPROM (%)

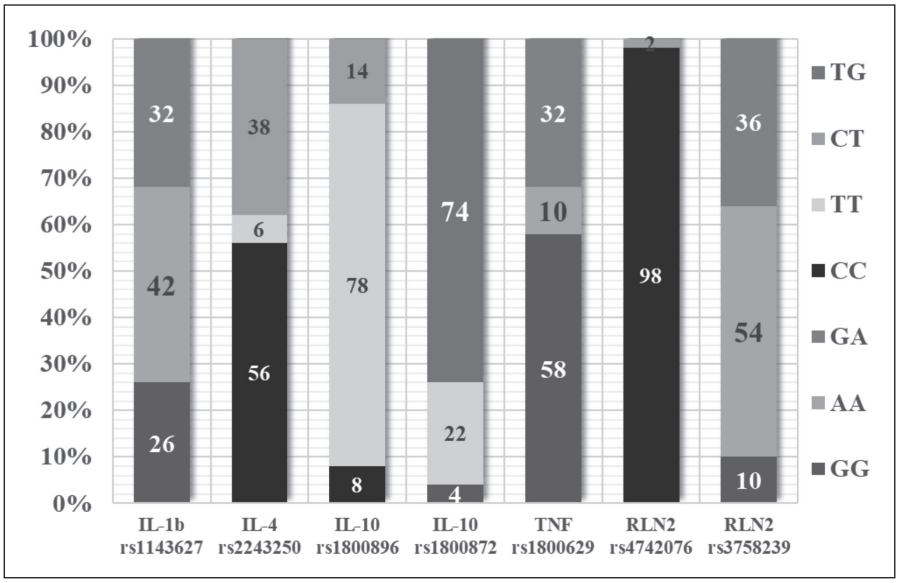

Fig 2. Frequency of genotypes distribution in control group (%)

understand the complexity of PPROM and PTL, further approaches using high-throughput genome sequencing methods such as genome wide association (GWAS), whole exome sequencing (WES) studies are essential.

Toll-like receptors (TLR)-2 and TLR-4 are innate immune receptors that recognize the microorganisms most frequently involved in amniotic cavity infections, which are associated with activating the inflammatory response at the maternal-fetal interface during PTB [12, 22, 23]. Transcriptional profile of innate and adaptive immunity genes in key tissues depending on the term of manifestation PPROM is shown in Table 2. The range of all obtained values of the relative normalized expression of TLR2 gene in the placenta of 0.79-163.44 (median – 31.06), in the fetal membranes – 1.1-126.06 (median – 10.22). The placement of all obtained values compared to mRNA expression of the TLR4 gene was lower than the TLR2 in the placenta, which was 0.39-43.85 (median – 7.74) and higher in the fetal membranes – 0.18-216.01 (median – 40.04).

The range of all obtained values of the relative normalized expression of pro-inflammatory IL1β gene mRNA in the placenta was 0.61-227.93 (mean – 25.08), in fetal membranes – 1.23-139.24 (mean – 23.83). The range of relative normalized expression of mRNA of the IL-17A gene in the placenta was 0.04-62.77 (mean – 5.69), in fetal membranes – 0.36-130.67 (mean – 19.31).

We identified significant decreased expression of FoxP3 (Treg) in women with PTB, induced PPROM compared with controls. We observed an 8.33-fold decreased expression in FoxP3 in decidua, especially in 31-32 weeks of PPROM manifestation (27.03-fold). In amniotic membranes a similar trend of reduction of FoxP3 expression was found, overall level decreased in 2.33 times, especially in 31-32 weeks of PPROM manifestation (10.64-fold).

The mRNA expression of TLR4 in the fetal membranes is significantly higher (40.04-fold) in patients with PTB, associated PPROM than in control group. In 19.31 times IL17A and 24.43 times RoRγt (Th17) higher transcriptional activity was present in membranes compared with

Table 2. Transcriptional profile of innate and adaptive immunity genes in key tissues depending on the term of manifestation PPROM (Mean (L-H))

Gene / local level	26-34 weeks n=30	26-30 weeks n=6	31-32 weeks n=10	33-34 weeks n=14
TLR2 placenta	31,06 (0,79-163,44)	46,31 (0,79-163,44)	37,74 (6,91-149,60)	21,22 (1,59-52,83)
TLR2 *membranes*	10,22 (1,1-126,06)*	3,27 (2,32-5,49)*	5,99 (1,1-28,88)*	15,61 (1,39-126,06)*
TLR4 *placenta*	7,74 (0,39-43,85)	11,09 (1,82-26,43)	3,08 (0,40-6,30)	10,00 (0,39-43,85)
TLR4 *membranes*	40,04 (0,18-216,01)*	34,38 (0,69-133,4)*	24,77 (0,53-152,97)*	53,53 (0,18-216,01)*
IL1β *placenta*	25,08 (0,61-227,93)	47,13 (1,43-227,93)	16,49 (0,61-132,74)	23,21 (1,63-91,02)
IL1β *membranes*	23,83 (1,23-139,24)	39,81 (4,47-77,11)	28,66 (2,79-139,23)*	12,85 (1,23-38,32)*
IL17A *placenta*	5,69 (0,04-62,77)	3,59 (1,15-5,62)	3,05 (0,04-5,73)	4,16 (0,92-62,77)
IL17A *membranes*	19,31 (0,36-130,67)*	15,46 (3,3-27,54)*	17,36 (0,36-49,74)*	13,73 (1,64-130,67)*
FoxP3 (Treg) *placenta*	0,12 (0,008-2,01)	0,093 (0,018-0,229)	0,037 (0,013-0,088)	0,191 (0,08-2,01)
FoxP3 (Treg) *membranes*	0,38 (0,013-0,986)	0,512 (0,143-0,927)	0,094 (0,013-0,167)	0,49 (0,07-0,98)
T-bet (Th1) *placenta*	20,29 (0,63-376,73)	79,27 (2,36-376,73)	5,48 (1,65-14,55)	9,8 (0,63-98,19)
T-bet (Th1) *membranes*	16,91 (0,51-101,71)	27,32* (0,51-71,93)	10,04* (1,43-44,45)	17,35* (1,27-101,71)
RoRγt (Th17) *placenta*	4,77 (0,692-44,41)	3,61 (1,25-6,14)	3,24 (1,09-6,26)	6,4 (0,69-44,41)
RoRγt (Th17) *membranes*	24,43* (0,75-141,89)	63,44* (6,26-141,89)	19,75* (0,75-87,68)	13,83* (0,86-100,93)

Note: * – statistically significant differences p <0,05 (Mann-Whitney U test) compared to the expression level of the studied gene in the placenta

term deliveries This may be reflective of the inflammatory and immune response that occurs as a breakdown in maternal-fetal tolerance or inflammation related to PTB. Our results shed light on potential mechanisms by which mRNAs may play a role in mediating systemic inflammatory response in pregnant women that deliver prematurely.

The modern molecular genetic method of research has allowed to reveal key immunoregulatory components of development of PROM at the local level during the 26-34 weeks' gestation period in the form of an expression violation of the receptors of innate immunity and consequently cell pool disbalance of T-helpers Th1/ Th17/ Treg.

CONCLUSIONS

1. There is evidence suggests that the nature and intensity of an inflammatory response in women with PTB induced PPROM is under genetic control. Among Zaporizhzhia population, combination of IL4 (rs2243250), IL10 (rs1800896 and rs1800872), RLN2 (rs4742076 and rs3758239) supports the role for functional polymorphisms in immunoregulatory genes in the susceptibility to PTL, associated with PPROM.
2. Marked increased transcriptional activity of components of innate (TLR2, TLR4), adaptive (Th1, Th17) immune system and conversely decreased expression of Treg (FoxP3) in the maternal-fetal interface are involved in immune pathways of PTB and contribute in the fetal inflammatory response syndrome.

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ORIGINAL ARTICLE
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CLINICAL AND SPIROGRAPHIC FEATURES OF BRONCHIAL ASTHMA IN SCHOOLCHILDREN DEPENDING ON THE DIFFERENT REGIMENS OF BASIC ANTI-INFLAMMATORY THERAPY

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ABSTRACT

The aim: To study the clinical and spirographic features persistence of the bronchial asthma in schoolchildren against the background of the alternative daily doses of inhaled corticosteroids to increase the effectiveness of anti-inflammatory therapy for this disease.

Materials and methods: A complete comprehensive clinical-paraclinical examination of 65 schoolchildren with persistent asthma was conducted. According to the average daily dose of inhaled corticosteroids (ICS) the patients were divided into two clinical groups. The first (I) group consisted of 46 children who received ICS in the regimen of low-to-medium equipotent doses (253.95±9.98 μg per day), and the second (II) comparison group was formed of 19 patients who controlled the pBA using high doses of ICS (494.74±5.56 μg per day).

Results: The patients of the I clinical group compared to patients of the II group have a higher risk of the mild bronchial obstructive syndrome during asthma attacks. In assessing the level of control of persistent bronchial asthma using the CIA-scale, it was found that in II group cases of the controlled course of the disease were observed almost two times less than in children of the I group of comparison. In conducting spirography in children of comparison groups, it was shown that the ratio of indices of bronchospasm (FEV_1/FVC) was worse in patients receiving high doses of ICS.

Conclusions: So, characteristic clinical feature of asthma controlled by high doses of ICS is more severe nature of bronchial obstructive syndrome during the period of exacerbation (OR=1.9-3.0). In the management of persistent bronchial asthma, the Gensler index which has high specificity (94.4%) and accuracy (92.2%) should be used for disease control verification.

KEY WORDS: bronchial asthma, schoolchildren, basic anti-inflammatory therapy, comprehensive clinical-paraclinical examination

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INTRODUCTION

Current clinical studies prove that bronchial asthma (BA) can be well-controlled in most of the pediatric patients, but practice demonstrates that the uncontrolled course of the disease occurs every so often, and prolonged maintenance of complete control over the disease remains an unattainable goal for most of children [1]. Inhaled corticosteroids (ICS) have a leading role in the treatment of asthma both at the stage of achieving and maintaining control over the clinical symptoms of the disease. In case of an insufficient control over the clinical asthma symptoms when using low-to-medium doses of ICS [2], optimization of the therapeutic tactics can be carried out in several ways: either an addition of a leukotriene modifier or a long-acting beta-agonist, or increasing the dose of inhaled corticosteroids [3].

To date there are multiple scientific disputes with no consensus on whether the low and medium doses of ICS are capable of causing side effects similar to those of systemic corticosteroids (SCS), in particular, retardation of bone growth, changes in carbohydrate metabolism, adrenal gland supression.

In his review B. Lipworth [4] shows the dose dependence of ICS side effects. However, a comparison of the side effects of different drugs should be made cautiously, as different methods of evaluation were used in various studies. Therefore, when asthma stabilization is achieved, it is always advisable to taper glucocorticosteroids to the minimum effective dose in order to reduce the likelihood of systemic effects and optimize the benefit/risk ratio.

High doses of ICS are recommended for the treatment of patients with resistant asthma, which is poorly controlled by the medium doses of ICS even in combination with other drugs for the standart asthma therapy. However, the use of high-dose ICS is associated with the development of systemic side effects, and a number of researchers believe this thesis is not an issue for discussion anymore [5].

Therefore, as the use of ICS remains the recommended method of asthma managemen for all the patients, these drugs should always be used in the minimum effective dose in accordance with the severity of the persistent asthma, since an insufficient control and frequent exacerbations of asthma are accompanied by an increased load on the child organism of SCS, which have multiple side effects.

THE AIM

To study the clinical and spirographic peculiarities of the bronchial asthma persistence in schoolchildren against the background of the alternative daily doses of inhaled corticosteroids to increase the effectiveness of anti-inflammatory therapy for this disease.

MATERIALS AND METHODS

A cohort of 65 school-age children with persistent bronchial asthma (pBA) was created by random method at the pulmonology and allergology Department of the Municipal Medical Establishment "Chernivtsi Regional Children's Clinical Hospital". Based on the informed consent obtained from parents, patients have been subjected to a comprehensive clinical-paraclinical examination.

The average age of the patients was 11.43±0.39 years, there were 81.54% of boys, and respectively – 18.46% of girls (P<0.001). The average age at which the pBA debuted was 2.09±0.09 years, so most of children had an early onset of disease.

Classification and management of pBA used according to the protocol of diagnosis and treatment of the disease in children [6]. One third of children (32.31%) had severe persistent asthma, 61.54% had moderate persistent asthma, and only 6.15% of patients had mild pBA. Two thirds of patients (60.0%) had an atopic form of pBA, and the rest of the children had a mixed form of asthma.

The equipotent doses of inhaled corticosteroids (ICS) received by patients as a component of the basic asthma treatment were determined according to current international guidelines and recommendations [7], distinguishing the low-dose, medium-dose and high-dose regimen of these drugs. Thus, the fewest number of children (9.23%) received low doses of ICS, the largest number (61.54%) – medium doses of drugs, and the rest of patients (29.23%) received high doses of ICS.

According to the average daily dose of ICS received by the examined children as a part of the basic treatment, patients were divided into two clinical groups for comparison. The first (I) group consisted of 46 children who received ICS in the regimen of low-to-medium equipotent doses (253.95±9.98 μg/day on average), and the second (II) comparison group was formed of 19 patients who controlled the pBA using high doses of ICS (494.74±5.56 μg/day). Thus, beclomethasone in the metered dose inhaler was given to 28.3% of patients from the I group and 57.9% of children from the II group (Pφ<0.05), budesonide – to 15.2% and 15.8% of patients respectively (Pφ>0.05), fluticasone – to 56.5% and 26.2% of children from the I and II group respectively (Pφ<0.05).

Groups were comparable by the main clinical characteristics. Thus, the fraction of boys was 80.4% in the I group and 84.2% in the II group, fraction of girls – 19.6% and 15.8% respectively (Pφ>0.05 in all cases). The fraction of the urban residents among the patients of the I group was 52.2%, the villagers – 47.8%. The distribution in the II group of comparison was 63.2% (Pφ>0.05) and 36.8% (Pφ>0.05) respectively. There were no significant differences in the severity of the asthma persistence: mild persistence was observed in 6.5% of cases in the I group and in 5.3% of patients from the II group, moderate persistence was diagnosed in 63.0% and 57.9% respectively, and severe – in 30.4% and 36.8% of observations in the I and II group respectively (Pφ>0.05 in all cases). The atopic form of pBA was registered in 60.9% of the I group patients and in 57.9% of cases in the II group (Pφ>0.05), and mixed form – in 39.1% and 42.1% of cases respectively (Pφ>0.05).

The severity of the bronchial obstruction syndrome (BOS) was assessed upon arrival at the hospital during the exacerbation period of asthma using a score scale [8]. An increase in score reflected the intensification of BOS manifestations, and the difference between the initial and an actual score reflected a degree of bronchial disobstruction. The evaluation of asthma control was carried out using a clinical and instrumental assessment scale (CIA) [9], according to which 10 and below points correspond to controlled asthma, 11-16 points – to partially controlled disease, and above 17 points – to uncontrolled variant of BA.

The statistical analysis of the obtained results was performed using the Statistica 6.0 software (StatSoft, USA). Student's t-criterion (Pt) was used to measure the differences between means, and Fisher's angular transformation criterion (Pφ) was used for comparision of percentage data. Differences were considered significant with Pt/Pφ<0.05.

The evaluation of the diagnostic value of spirographic tests was conducted with the determination of sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) of the results, and posttest probability (PP) for the positive and negative results. In all cases, a 95% confidence interval was determined (95% CI). To conduct the population analysis the relative risk (RR), attributable risk (AR), and the odds ratio (OR) with the calculation of their confidence intervals (95% CI) were estimated.

RESULTS AND DISCUSSION

An analysis of the level of pBA control in children of the clinical groups did not identify the fundamental differences, and results of the estimation using the CIA-scale in general complied with the criteria of the partially controlled disease (Table 1).

Despite the absence of significant differences between the average total scores according to results of the clinical assessment of pBA control, the incidence of controlled asthma decreased 2-fold among the patients of the II group compared to children of the I group (Table 2).

Thus, in contrast to patients receiving high doses of ICS as a basic treatment of pBA, the risk of controlled pBA increased 2-fold in representatives of the I group: OR=2.1 (95% CI 0.52-8.44), RR=1.79 (95% CI 1.32-2.43), AR=0.13. Consequently, it is assumed the existence of a "difficult to treat asthma" phenotype among the patients of the II group [10].

This assumption was partly confirmed by the results of the clinical assessment of the severity of asthma exacer-

Table 1. Comparative assessment of the clinical signs of asthma control in children of the comparison groups during the period between exacerbations (M±m)

	Indices of bronchial asthma control (points)	Clinical groups		P value
		I group (n=46)	II group (n=19)	
Clinical symptoms of disease	Daytime symptoms of disease	2.57±0.14	2.37±0.23	>0.05
	Nightime symptoms of disease	1.57±0.12	1.53±0.19	>0.05
	β_2-agonists when needed	1.74±0.13	1.63±0.21	>0.05
	Limitation of physical activity	2.46±0.12	2.37±0.18	>0.05
	Frequency of hospitalizations	1.87±0.17	2.08±0.13	>0.05
	Frequency of exacerbations	2.61±0.09	2.42±0.16	>0.05
	Unplanned visit to an allergist	0.91±0.11	0.84±0.18	>0.05
	Total score	13.72±0.62	12.95±1.06	>0.05

Table 2. Frequency (%) of the levels of asthma control in children of the clinical comparison groups (P±m)

Clinical groups (number of patients)	Controlled pBA	Partially controlled pBA	Uncontrolled pBA
I group (n=46)	28.26±6.64	45.65±7.34	26.09±6.4
II group (n=19)	15.79±8.37	63.16±11.07	21.05±9.35
Pt value	>0.05	>0.05	>0.05

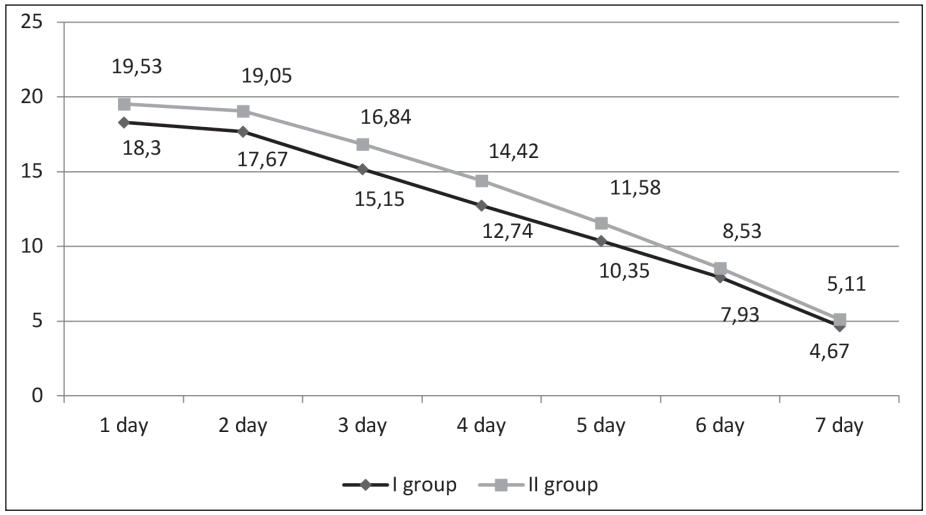

Fig. 1. Dynamics of the severity of bronchial obstruction syndrome in children of the comparison groups during hospital stay (points)

bations in children of the comparison groups, reflecting a steady tendency to more severe BOS in representatives of the II clinical group (Fig. 1).

The risks of more pronounced bronchial obstruction syndrome during the acute asthma exacerbation in patients of the II clinical group compared to patients who received low-to-medium doses of ICS as a basic anti-inflammatory therapy are presented in Table. 3

Thus, in children with pBA who received high doses of ICS as a basic therapy, the risk of the severe asthma exacerbation is increased, and, therefore, the adjuvant therapy should be provided to this patients maximally rapidly and to the fullest extent.

Accordingly, as shown in Table 4, patients of the I clinical group compared to patients of the II group have a higher risk of the mild bronchial obstructive syndrome during asthma attacks.

In our opinion, the identified patterns could be explained either by the peculiarities of the trigger factors which contributed to the asthma exacerbation or by the prevalence of the neutrophilic inflammatory process in the respiratory tract of patients from the II clinical group, which, as is known, increases asthma severity [11].

During the period between exacerbations against the background of discontinuation of drugs that may affect the results, a spirographic examination was performed. It included initial spirography, a test with dosed physical activity and inhalation of a short-acting β_2-agonist (salbutamol, 200 μg) with measurement of the forced expiratory volume in 1 s (FEV_1), forced vital capacity of lungs (FVC),

Table 3. Risks of persistent pronounced airway obstruction against the background of high doses of ICS compared to patients of the I group

Day of treatment (distribution point, score)	Attributable risk	Relative risk (95% CI)	Odds ratio (95% CI)
1st (> 18 points)	0.27	1.8 (1.4-2.3)	3.04 (1.7-5.5)
3rd (> 16 points)	0.17	1.4 (1.0-1.9)	1.9 (1.1-3.3)
4th (> 15 points)	0.19	1.4 (0.9-2.2)	2.1 (1.2-3.9)

Table 4. Risks of the mild bronchial obstructive syndrome against the background of low-to-medium doses of ICS compared to patients of the II group

Day of treatment (distribution point, score)	Attributable risk	Relative risk (95% CI)	Odds ratio (95% CI)
1st (< 19 points)	0.21	1.5 (1.1-2.1)	2.36 (1.3-4.2)
2nd (<19 points)	0.14	1.3 (1.0-1.7)	1.7 (1.0-3.0)
2nd (< 17 points)	0.18	1.4 (0.8-2.4)	2.1 (1.1-4.2)
3rd (< 14 points)	0.21	1.4 (0.7-2.9)	2.4 (1.1-5.2)

Table 5. Average indices of the spirographic examination in children of the comparison groups (M±m)

Spirographic index	I group (n=49)	II group (n=16)	Pt value
FEV1 (%)	84.69±2.59	81.82±6.33	>0.05
FVC (%)	90.82±2.49	97.46±4.58	>0.05
FEV1/FVC	0.93±0.03	0.82±0.02	=0.05
BSI FEV1 (%)	10.92±2.02	8.82±3.90	>0.05
BDI FEV1 (%)	14.84±2.52	14.98±4.83	>0.05
BLI FEV1 (%)	24.1±3.27	20.74±6.08	>0.05

FEV_1/FVC ratio, indices of bronchospasm (BSI) and bronchodilation (BDI), and bronchial lability index (BLI).

The comparative indices of the spirographic examination in children of the clinical comparison groups are presented in Table 5.

Despite the absence of the significant differences between the FEV_1 and FVC in the comparison groups, the FEV_1/FVC ratio was worse in patients receiving high doses of ICS. Based on the fact that the value of this ratio less than 70% indicates a marked impairment of bronchial passability [12], we have studied the peculiarities of its distribution within the clinical comparison groups. It was established that the value of the Gensler index (modified Tiffeneau index) below 70% occurred in the I group with a frequency of 5.6%, while in the II group it was observed in 27.3% of patients ($P\varphi<0.01$). Therefore, in patients receiving high doses of ICS, there was an increased risk of impaired bronchial passability: OR=6.4 (95% CI 2.4-16.7), RR=1.9 (95% CI 0.8- 4.6), AR=0.40. Apparently, it could be explained by a more pronounced inflammatory process in bronchi with the probable development of their remodeling, which is not consistent with the assessment of asthma severity and disease control from the clinical point of view.

It should be noted that in children with uncontrolled asthma the Gensler index was below 70.0% in one-third of cases, while in patients with controlled and partially controlled pBA – only in 5.56% of observations. As a result, patients with unsatisfactory control of pBA (over 17 points according to CIA scale) had high chances of the impairment of bronchial passability even during the period of clinical well-being: OR=8.5 (95% CI 3.3-22.0), RR=2.07 (95% CI 1.0-4.9), AR=0.44.

Hence, obtaining the values of Gensler index which do not reach 70.0% can be used as an additional highly specific test for the verification of the unsatisfactory control of pBA: the sensitivity of this test is 33.3% (95% CI 24.16-43.46%), the specificity is 94.44% (95% CI 87.95-98.05%), the positive predictive value is 85.69% (95% CI 70.66-94.86%), the negative predictive value – 58.61% (95% CI 50.58-66.31%), test accuracy – 92.2% (95% CI 67.95-92.0%). With positive values, posttest probability (+) increases by 25.65%, and with a negative result – posttest probability (-) decreases by 8.61%.

CONCLUSIONS

1. A possible cause of the patients insensitivity to low and medium doses of ICS is the increased severity and neutrophilic nature of inflammation, resulting in the persistence of bronchial ventilation disturbances in the post-exacerbation period (OR=8.5).
2. Characteristic clinical feature of asthma controlled by high doses of ICS is the more severe nature of bronchial obstructive syndrome during the period of exacerbation (OR=1.9-3.0).
3. In the management of persistent bronchial asthma, the Gensler index which has high specificity (94.4%) and accuracy (92.2%) should be used for disease control verification.

4. When obtaining on the results of spirographic examination the values of the Gensler index below 70.0% the amount of the basic anti-inflammatory therapy should be reviewed using the "step up" principle.

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ORIGINAL ARTICLE
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ISCHEMIC MITRAL REGURGITATION: PROBLEM EXTENT IN CARDIOVASCULAR SURGERY CLINIC

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ABSTRACT

The aim of the study was to evaluate the prevalence, etiology, mechanisms and severety of all cases of MR, including IMR, in the patients population with suspected or proved CAD before coronary arteries evaluation and surgery or intervention.

Materials and methods: The study prospectively included 370 patients with verified or suspected (angina pectoris suspicion or manifested CHF) CAD of all clinical forms (stable, unstable, post-AMI, prior revascularization, etc.). MR evaluation by TTE included examining type of MR (organic or functional, Carpentier type) and its severity by vena contracta (VC) width and establishing effective regurgitant orifice (ERO) and regurgitant volume (RVol) by PISA method according to existing guidelines. Additional criteria (left atrium (LA) dimensions, LV diastolic filling and filling pressure markers, pulmonary veins flow and secondary pulmonary hypertension and right chambers involvement, etc.) were also widely used.

Results: Majority of all patients were men – 280 out of 370 pts (75.7%). Mean age of pts was 62.4±8.96 years, and men were in general significantly younger (61.5±9.2 vs. 65.3±7.6 years, p=0.0004). 145 (39.2%) pts had verified AMI previously (126 men and 19 women, p<0.0001). 22 (5.95%) pts previously underwent CABG surgery (19 men and 3 women, p<0.0001), and 99 patients – PCI with coronary stenting (81 men and 18 women, p<0.0001). 42 (11.3%) pts proved to have no significant CAD as per CAG results: 24 (57.1%) pts had no significant cardiac pathology at all, 12 (28.6%) pts had uncomplicated essential hypertension, 5 (11.9%) pts had non-coronary dilated cardiomyopathy (DCM) and 1 (2.4%) pt had non-obstructive hypertrophic cardiomyopathy (HCP) with clean coronary arteries. In patients with CAD MR is a frequent finding (up to one-half of the studied population) regardless of gender. Predominant majority (84.1%) of MR according to TTE findings is mild (grade I), without significant influence upon heart load and remodeling, and, thus, requiring no additional surgeon interventions. Most of cases of such pathological MR resolved after myocardial revascularization or sustained without dynamics being physiological (13.2% of all MR cases). Most of moderate-to-severe MR cases were classified as IMR, being accompanied by appropriate LV remodeling features, requiring revascularization and/or mitral valvuloplasty or replacement (all cases of severe IMR or organic MR). Correlations results of MR ERO and RVol with certain heart chambers remodeling and load indices seem to make them a "gold standard" for MR severity evaluation, especially in CAD patients with potential indications for MV surgery that should accompany surgeon revascularization, as it is widely accepted today.

Conclusions: In the patients with various forms of chronic and acute CAD IMR is a frequent finding, but the majority of MR cases are mild and requiring no additional interventions. No gender difference in MR prevalence were found. Severe MR cases in all CAD patients population are rather rare (below 10%), but always require surgical repair. Moderate-to-severe IMR is the most frequent (approximately 75%) etiology for significant MR in CAD patients, requiring close TTE follow-up for optimal intervention timing on the basis of left and right chambers remodeling and load indices. MR ERO and RVol are trustworthy quantitative MR severity indices, significantly correlating with main LV and LA remodeling and load indices, as well as with some secondary right chambers overload predictors.

KEY WORDS: coronary arteries disease, ischemic mitral regurgitation, transthoracic echocardiography

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INTRODUCTION

Chronic ischemic mitral regurgitation (IMR) is a subtype of secondary mitral regurgitation (MR) and a complication of coronary arteries disease (CAD). It is a pathophysiological phenomenon of insufficient mitral cusps coaptation mostly due to global or local left ventricle (LV) geometry impairment with mitral valve (MV) apparatus deformation. Most of IMR cases are secondary to LV geometry alterations [1, 2] in the patients with ischemic cardiomyopathy (ICM), chronic or acute (postinfarction), while primary organic cases due to mitral chords rupture during acute myocardial infarction are relatively rare [3]. Mechanisms of IMR were widely described [1,2,4,5], being usually referred to as type IIIb Carpentier [1]. Although IMR is associated with significant (>70%) of at least one trunk coronary artety [4], the term IMR itself not necessarily include real current myocardial ischemia, but often characterizes certain clinical situation of postischemic changes, leading to global or regional pathological remodeling progression [5]. Detailed studies with thorough echocardiography (EchoCG) evaluation spotted various LV remodeling prognostic markers of reverse remodeling and IMR and congestive heart failure (CHF) progression [1,2,6].

Overall prevalence of all causes MR in Europe remains high, being the second most common valve disease that affect up to one third of population [4]. IMR is described as the second most common cause of MR (25%) after degenerative (60%) and far ahead of rheumatic MR (12%),

Table 1. General characteristics of study group

• Total – 370 pts
Men – 280 (75.7%)
Women – 90 (24.3%), p<0.0001
• Age – 62.4±8.96 yrs.
Men – 61.5±9.2
Women – 65.3±7.6, p=0.0004
• Prior AMI – 145 (39.2%)
Men – 126 (86.9%)
Women – 19 (13.1%), p<0.0001
• Prior CABG – 22 (5.95%)
Men – 19 (86.4%)
Women – 3 (13.6%), p<0.0001
• Prior PCI / coronary stenting – 99 (26.8%)
Men – 81 (81.8%)
Women – 18 (18.2%), p<0.0001
• CAD not verified by CAG results – 42 (11.3%)
Healthy heart – 24 (57.1%)
Uncomplicated essential hypertension – 12 (28.6%)
Non-coronary DCM – 5 (11.9%)
Non-obstructive HCM – 1 (2.4%)

progressively increasing recently. Up to 40% of patients after AMI are also diagnosed with IMR by EchoCG [7].

Surgery remains the basis for IMR treatment in most cardiac surgery units due to unsatisfactory medication treatment results and significant prognosis worsening in case of marked IMR. Namely, IMR is associated with three-times increase of clinically significant CHF and 1.6 times mortality increase in the next 5 years [8]. Mortality rates over 5 – 7 years follow-up in cases of conservative treatment remains high, constituting 60 – 100% depending on IMR severity, CAD severity and LV remodeling advance [6]. There is a strong evidence that CABG or PCI themselves with or without MV annuloplasty lead to significant reverse left chambers remodeling and significantly improve prognosis in CAD patients [9], unless MR is severe and/or organic, definitely requiring surgeon repair. Still, today we lack detailed prospective evidence and focus of international guidelines on IMR.

THE AIM

The aim of the study was to evaluate the prevalence, etiology, mechanisms and severety of all cases of MR, including IMR, in the patients with suspected or proved CAD, who were to undergo possible revascularization.

MATERIALS AND METHODS

The study was approved by local ethics committee. The materials, used in the study, do not violate the principles of bioethics and can be published. All patients participating in the study gave their consent and signed an approved informed consent form (ICF).

The study prospectively included 370 patients with verified or suspected (angina pectoris suspicion or manifested CHF) CAD of all clinical forms (stable, unstable, post-AMI, prior revascularization, etc.). No exclusion criteria for MR evaluation were set. During and after diagnostic work-up and coronary angiography (CAG) and/or revascurarization standard none of the patients was diagnosed with perioperative myocardial infarction. Medication treatment in all the post-infarction patients included aspirin and/or clopidogrel, statins, beta-blockers, ACE inhibitors or sartans, and nitrates, if indicated. All patients underwent transthoracic EchoCG (TTE) prior any interventions and after, if indicated. Program of the study included X-ray contrast CVG and Doppler TTE with LV myocardial speckle-tracking (STE).

CAG was conducted and interpreted by trained physicians A 50% or more reduction of the luminal diameter in 2 orthogonal projections of a major coronary artery or one of its major branches or a bypass graft was considered to be significant for CAD.

During TTE with STE recordings and calculations of different parameters, including LV chamber volumes and EF, were performed according to existing Guidelines [10]. MR evaluation included examining type of MR (organic or functional, Carpentier type) and its severity by vena contracta (VC) width and establishing effective regurgitant orifice (ERO) and regurgitant volume (RVol) by PISA method according to existing guidelines [1,2]. Additional criteria (left atrium (LA) dimensions, LV diastolic filling and filling pressure markers, pulmonary veins flow and secondary pulmonary hypertension and right chambers involvement, etc.) were also widely used [1,2,4].

Comparison of different parameters was performed using multiple regression analysis with 95% confidence interval and correlation analysis.

RESULTS AND DISCUSSION

Majority of all patients were men – 280 out of 370 pts (75.7%). Mean age of pts was 62.4±8.96 years, and men were in general significantly younger (61.5±9.2 vs. 65.3±7.6 years, p=0.0004). 145 (39.2%) pts had verified AMI previously (126 men and 19 women, p<0.0001). 22 (5.95%) pts underwent CABG surgery (19 men and 3 women, p<0.0001), and 99 patients previously underwent PCI with coronary stenting (81 men and 18 women, p<0.0001). 42 (11.3%) pts proved to have no significant CAD as per CAG results: 24 (57.1%) pts had no significant cardiac pathology at all, 12 (28.6%) pts had uncomplicated essential hypertension, 5 (11.9%) pts had non-coronary dilated cardiomyopathy (DCM) and 1 (2.4%) pt had non-obstructive hypertrophic cardiomyopathy (HCP) with clean coronary arteries (Table 1).

182 (49.2%) pts had MR according to TTE results with no significant difference between genders (142 (50.7%) men vs. 40 (44.4%) women, p=0.30).

According to etiology MR was classified as:

- trivial (physiological) MR – minimal regurgitant flow on intact MV in the patients with normal left chambers and no regional contractility abnormalities or ischemic remodeling signs;
- ischemic MR (IMR) – any severity MR in the patients with verified CAD and obvious ischemic LV and/or LA remodeling (dilation, regional wall contraction abnormalities, obvious papillary muscles dysfunction, MV tethering and restriction, etc.);
- sunctional secondary MR – left chambers and mitral ring dilation with MV tenting in the absence of verified CAD due to DCM, atrial fibrillation, etc.);
- organic (primary) MR of any etiology (Barlow disease, rheumatic, flail MV, etc.).

Organic MR was rare in the studied group – 9 (4.95%) cases with no gender differences (4 men vs. 5 women, p=0.64). Of them only 4 pts. had verified CAD, of which 2 had stable insignificant CAD (50% LAD in one case and patent stents in the other), while 2 other required CABG surgery due to multi-vessel disease, but had obviously organic etiology of MR (flail P2 segment due to Barlow disease and mild rheumatic MR combined with mild stenosis) (Table 2). Only 3 patients required urgent MV surgery due to severe decompensated MR with clinically significant CHF due to flail MV. Therefore, all these cases of obviously non-ischemic primary MR were no further analyzed.

Table 2. Etiology and severity of primary organic MR in the studied group.

Severity (Total 9 (4.95%) cases)
• Grade I (mild) – 4 (44.5%)
• Grade II (moderate) – 2 (22.2%)
• Grade III (severe) – 3 (33.3%)
Etiology
• Rheumatic – 3 (33.3%)
Combined mitral without prevalence grade 1 (mild)
Combined mitral with MR prevalence grade 2 (moderate)
• Barlow disease (MV prolapse) – 6 (66.7%)
With MR grade 1 (mild) – 1 (11.1%)
With MR grade 2 (moderate) – 2 (22.2%)
Flail MV (chords rupture) – 3 (33.3%)

Table 3. Correlation of MR severity indices with left and right chambers function, remodeling and overload markers.

Index	MV ERO	MV RVol	Significance between two correlation coefficients
LV iEDD, cm/m^2	**r=0.222, p=0.0024**	r=0.347, p<0.0001*	p=0.20
LV iEDV, ml/m^2	**r=0.193, p=0.0085**	**r=0.353, p<0.0001**	p=0.10
LV iESV, ml/m^2	r=0.111, p=0.13	r=0.234, p=0.0014	p=0.23
LAs, cm	r=0.242, p=0.0009	r=0.481, p<0.0001	p=0.0091
LAV, ml	r=0.354, p<0.0001	r= 0.639, p<0.0001	p=0.0003
iLAV, ml/m^2	r= 0.390, p<0.0001	r= 0.679, p<0.0001	p=0.0001
LV EF, %	r= – 0.02, p=0.77	r= – 0.10, p=0.16	p=0.45
E/A	**r=0.219, p=0.0028**	**r=0.404, p<0.0001**	p=0.052
E/E'	r=0.093, p=0.21	r=0.077, p=0.30	p=0.88
LV diastolic dysfunction grade	**r=0.192, p=0.009**	**r= 0.344, p<0.0001**	p=0.12
PA SBP, mm Hg	r=0.104, p p=0.16	**r= 0.244, p=0.0009**	p=0.17
PA Mean BP, mm Hg	r=0.087, p=0.25	**r=0.225, p=0.0021**	p=0.18
RA short, cm	r=0.073, p=0.33	**r=0.176, p=0.017**	p=0.32
RA long, cm	r=0.005, p=0.94	r=0.011, p=0.88	p=0.96
iRAV, ml/m^2	r=0.112, p=0.13	**r=0.198, p=0.0007**	p=0.41
RV basal EDD, cm	r=0.126, p=0.088	**r=0.227, p=0.002**	p=0.32
RV wall, cm	r=0.117, p=0.11	**r=0.24, p=0.001**	p=0.23
RV TAPSE, cm	r=0.117, p=0.11	r= – 0.03, p=0.72	p=0.16
RV S', cm/s	r= – 0.01, p=0.92	r= 0.023, p=0.75	p=0.76
IVC, cm	r= 0.125, p=0.09	**r= 0.176, p=0.017**	p=0.62
CVP, mm Hg	r= 0.07, p=0.34	r= 0.119, p=0.11	p=0.64
LV GLS, %	r= – 0.03, p=0.66	r= – 0.01, p=0.92	p=0.85
RV GLS, %	r= – 0.02, p=0.79	r= – 0.01, p=0.91	p=0.93
TR ERO, cm^2	**r=0.191, p=0.01**	**r=0.288, p<0.0001**	p=0.33
TR RVol, ml	**r=0.216, p=0.0033**	**r=0.323, p<0.0001**	p=0.28

* – bold – weak to moderate significant correlation and significant difference between correlation coefficients.

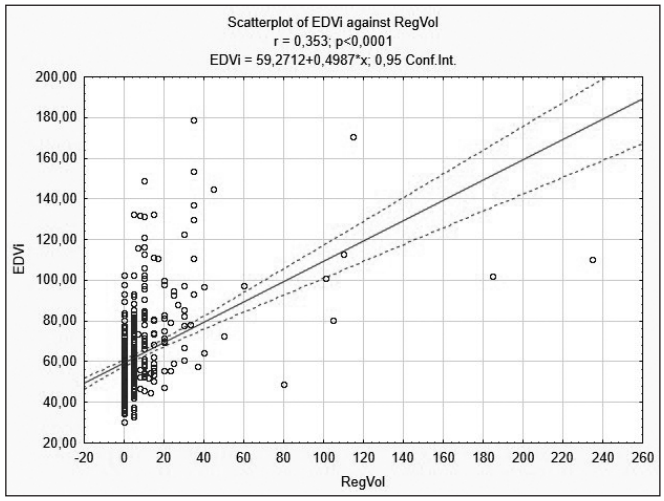

Fig. 1. Weak-to-moderate correlation between MR RVol and LV iEDV (r=0.353; p<0.0001).

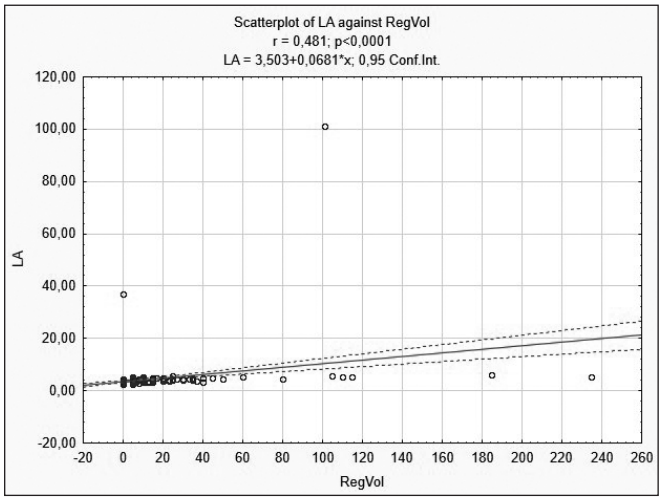

Fig. 2. Weak-to-moderate correlation between MR RVol and LA end-systolic diameter (r=0.481; p<0.0001).

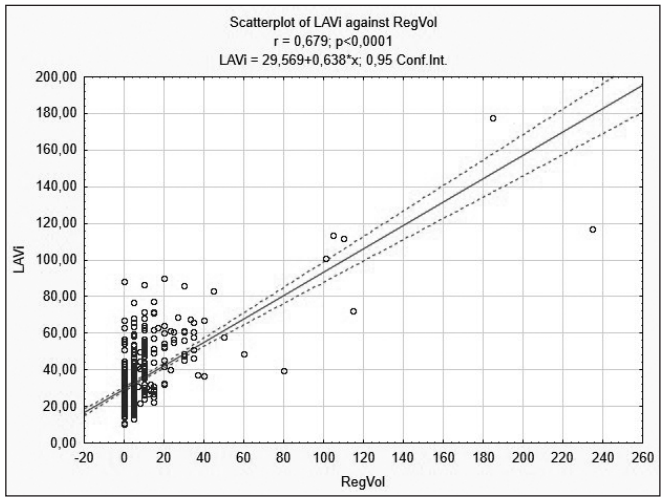

Fig. 3. Moderate correlation between MR RVol and iLAV (r=0.679; p<0.0001).

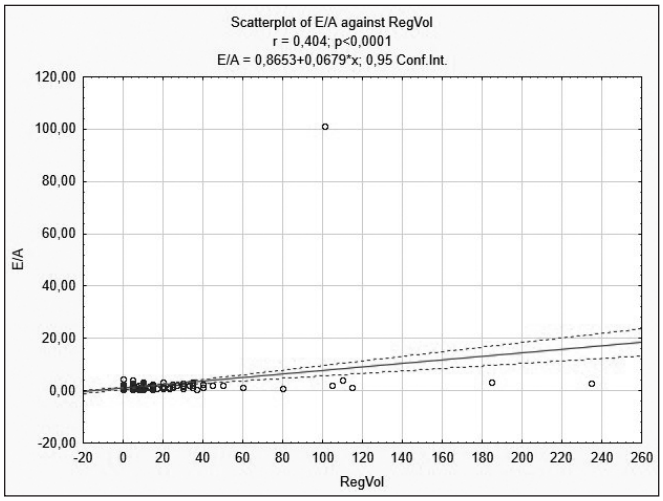

Fig. 4. Weak-to-moderate correlation between MR RVol and LV diastolic filling (E/A ratio) (r=0.404; p<0.0001).

Overwhelming majority of all registered MR cases were patients with mild (grade I) MR – 153 (84.1%) cases with no significant difference between genders (122 (85.9%) men vs. 31 (77.5%) women, p=0.060). Although women showed significantly higher quantitative extent of MR with higher ERO (0.17±0.54 vs. 0.08±0.04 cm^2, p=0.0058) and RVol (9.9±6.2 vs. 8.3±4.5 ml, p=0.0082), there was no gender differences in the etiology of mild MR. There were 24 (15.7%) cases of trivial physiological MR, 11 (7.2%) cases of secondary non-ischemic MR and 4 (2.6%) cases of mild primary organic MR (rheumatic or due to MV prolapse). The majority (114 (74.5%) cases) of mild MR (grade I) were qualified as ischemic MR in the patients with verified CAD and obvious ischemic LV and/or LA remodeling. Still, it did not require additional surgeon intervention, as well as moderate MR grade 2.

Significant (grades 2 and 3) MR was not so frequent.

There were only 16 (8.8%) cases of moderate (grade 2) MR, more frequent in women (20.0% vs. 5.6%, p=0.044) with no significant difference in ERO (0.2±0.00 cm^2) and RVol (31.4±4.2 ml). Majority of moderate MR was IMR – 12 (75.0%) cases.

There were only 13 (7.1%) cases of severe MR (grade 3) with ERO 0.59±0.40 cm^2 and RVol 86.9±62.8 ml. All cases were accompanied by marked symptomatic CHF, corresponding LV and LA overload and marked secondary pulmonary hypertension, and required surgery. Most of cases (9 (75.5% pts) were qualified as IMR with indications for CABG combined with valvuloplasty or MV replacement (MVR).

Both MR ERO and RVol showed to weakly to moderately but significantly correlate main LV and LA remodeling indices, especially RVol, which significantly correlated with indexed to body surface area (BSA) LV end-diastolic diameter (EDD) and volume (EDV), and especially with LA end-systolic diameter (LAs) and absolute and BSA-indexed LA volume (LAV and iLAV). Also, significant correlation between LV diastolic dysfunction grade and E/A ratio on one hand, and MR RVol, on the other. Correlation between MR RVol and right-sided overload indices (pulmonary artery (PA) systolic (SBP) and mean pressure and right ventricle (RV) and atrium (RA) diameters) were weak but significant, which shows that MR indices might also show prognostic about indices of pulmonary hyperten-

sion and right chambers load and function, including significant weak-to-moderate correlation with indiced of secondary tricuspid regurgitation (TR) (Table 3). In general, MR RVol correlated with LV and LA remodeling and load indices better than MR ERO (Fig. 1 – 4). No correlation was found between indices of MR severity and LV and RV systolic function indices, namely LV EF and LV and RV global longitudinal strain (GLS) by STE.

Also, we found no significant correlation between VC width and most of the studied heart chambers remodeling and load indices.

According to the results of our study, involving 370 patients with CAD MR is a frequent finding (up to one-half of the studied population) regardless of gender. Predominant majority (84.1%) of MR according to TTE findings is mild (grade I), without significant influence upon heart load and remodeling, and, thus, requiring no additional surgeon interventions. Most of cases of such pathological MR resolved after myocardial revascularization or sustained without dynamics being physiological (13.2% of all MR cases).

Most of moderate-to-severe MR cases were classified as IMR, being accompanied by appropriate LV remodeling features, such as postinfarction aneurysms, scars, marked regional contraction abnormalities, LA and mitral annulus dilation, LV global and regional dilation with MV tethering, requiring revascularization and/or mitral valvuloplasty or replacement (all cases of severe IMR or organic MR).

Correlations results of MR ERO and RVol with listed above heart chambers remodeling and load indices seem to make them a "gold standard" for MR severity evaluation, especially in CAD patients with potential indications for MV surgery, that should accompany surgeon revascularization, as it is widely accepted today. At the same time, VC as per our experience, is much less validated, and can remain only as empirical qualitative method, that should not be used for quantifying MR severity without other indices.

Prospective inclusion of patients in the absence of inter-observer variability (all TTE were performed by the same sonographer on one EchoCG machine) allowed to avoid typical inter-observer study limitations.

CONCLUSIONS

In the patients with various forms of chronic and acute CAD IMR is a frequent finding, but the majority of MR cases are mild and requiring no additional interventions. No gender difference in MR prevalence were found. Severe MR cases in all CAD patients population are rather rare (below 10%), but always require surgical repair. Moderate-to-severe IMR is the most frequent (approximately 75%) etiology for significant MR in CAD patients, requiring close TTE follow-up for optimal intervention timing on the basis of left and right chambers remodeling and load indices. MR ERO and RVol are trustworthy quantitative MR severity indices, significantly correlating with main LV and LA remodeling and load indices, as well as with some secondary right chambers overload predictors.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

REGULATION OF MICRORNA EXPRESSION LEVEL BY CHOLERETIC THERAPY IN FUNCTIONAL DISORDERS OF THE GALLBLADDER AND ODDI'S SPHINCTER IN CHILDREN

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ABSTRACT

The alm: To study the effect of choleretic therapy on the level of microRNA expression in functional disorders of the gallbladder and Oddi's sphincter in children.

Materials and methods: Fifty patients with functional disorders of the gallbladder and Oddi's sphincter who received standard therapy in combination with ursodeoxycholic acid, 20 patients – standard therapy without ursodeoxycholic acid, and 20 healthy children were examined. The level of expression of microRNA-378f, microRNA-4311, microRNA-4714-3p in the blood serum by the method of real-time polymerase chain reaction with reverse transcription according to the protocol TaqMan Gene Expression Assays was performed.

Results: It was demonstrated that the activity profile of microRNA-4714-3p was significantly lower in those examined with functional disorders of the gallbladder and Oddi's sphincter than in practically healthy children ($p<0.05$). After standard therapy combined with ursodeoxycholic acid in children with functional disorders of the gallbladder and Oddi's sphincter, the level of expression of microRNA-378f is significantly higher than before therapy (5.23±0.70 SU and 2.02±0.57 SU respectively) ($p<0.05$). Against the background of standard therapy with the addition of ursodeoxycholic acid or without it, the expression profile of microRNA-4714-3p in the blood serum in children with functional disorders of the gallbladder and Oddi's sphincter significantly decreased (1.93±0.58 SU and 1,14±0,53 SU respectively) ($p<0.05$).

Conclusions: Ursodeoxycholic acid in children with functional disorders of the gallbladder and Oddi's sphincter affects the activity of generation of gene regulators of the cellular mechanisms of microRNA-378f and microRNA-4714-3p.

KEY WORDS: The functional disorders of the gallbladder and Oddi's sphincter, microRNA, ursodeoxycholic acid, children

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INTRODUCTION

Scientific and practical studies of recent years indicate the wide prevalence of functional gastrointestinal disorders, as one of the most pressing problems of modern pediatric gastroenterology [1].

Functional disorders of the gallbladder and Oddi's sphincter are put down to functional disorders of the biliary system in children according to the Rome IV consensus the functional gastrointestinal disorders (2016) [1]. The acuteness of the problem of functional disorders of the gallbladder and Oddi's sphincter in Pediatric Gastroenterology is stipulated by the high prevalence and the possibility of transition to biliary system's organic pathology [2; 3]. The functioning of the gallbladder and Oddi's sphincteris is carried out due to the humoral effect of secretin, cholecystokinin [4]. Regulation of the production of these substances is performed by microRNA [5]. MicroRNA (miRNA, miR) are small non – coding RNA molecules of 21-23 nucleotides that regulate gene expression at the post-transcription level by RNA interference and indirectly they participate in protein synthesis, cell differentiation, and tissue [6].

In recent years, there is evidence of association of amplification or inhibition of microRNA generation with dysfunction and development of the biliary system's diseases [5; 7; 8].

Ursodeoxycholic acid (UDCA) is a tertiary bile acid that is formed in hepatocytes and intestinal cells. Ursodeoxycholic acid has hepatoprotective, anti-cholestatic, litholytic cytoprotective, antifibrotic, anti-apoptotic, antioxidant, immunomodulating effects. The results of numerous scientific studies confirm the positive therapeutic effect of ursodeoxycholic acid preparations in diseases of the digestive system [9; 10]. Recently, scientists have paid considerable attention to determining the effect of UDCA on the expression of various microRNAs [8; 11; 12]. However, the effect of ursodeoxycholic acid on the expression profile of microRNA in functional disorders of the gallbladder and Oddi's sphincter in children has not been determined yet.

THE AIM

Objective: to study the effect of choleretic therapy on the level of microRNA expression in functional disorders of the gallbladder and Oddi's sphincter in children.

MATERIALS AND METHODS

The comprehensive survey of 90 children aged 4 to 14 years was conducted according to the Rome IV consensus

the functional gastrointestinal disorders (2016) on the basis of the municipal institution "Dnipro city clinical hospital №1" Dnipro city Council, Dnipro city, Ukraine. 70 patients showed clinical signs of functional disorders of the gallbladder and Oddi's sphincter [1]. Verification of diagnosis and provision of medical care to patients were carried out in accordance with the order of the Ministry of Health of Ukraine dated 29.01.2013 № 59 "On approval of unified clinical protocols of medical care for children with diseases of the digestive system" [3]. The research was conducted in accordance with the principles of the Helsinki Declaration and with the informed consent of the patient's parents or guardians.

Patients with functional disorders of the gallbladder and Oddi's sphincter by simple randomization were divided into two groups. The first (1st, main) group was represented by 50 patients with functional disorders of the gallbladder and Oddi's sphincter. They received standard therapy in accordance with the provisions of the order of the Ministry of Health of Ukraine dated 29.01.2013 №59 in combination with ursodeoxycholic acid, which was prescribed at the rate of 10-15 mg/kg/day. The second (2nd) group (comparison group) included 20 patients with functional disorders of the gallbladder and Oddi's sphincter. They received standard therapy in accordance with the provisions of the order of the Ministry of Health of Ukraine dated 29.01.2013. № 59 without ursodeoxycholic acid. The course of treatment was 4 weeks. The effectiveness of treatment after completion of complex therapy for all children was evaluated. The third (3rd) group (control group) included 20 healthy children. Before treatment and after treatment, the molecular genetic study was conducted to determine the level of expression of microRNA-378f, microRNA-4311, microRNA-4714-3p in blood serum by real-time polymerase chain reaction with reverse transcription in children of groups 1 and 2 and once in children of group 3. It was performed according to the Taq Man Gene Expression Assays Protocol in the certified laboratory of the Department of General and molecular Pathophysiology of the Institute of Physiology after A. A. Bogomolets of NAS of Ukraine (Head – Doctor of Medical Science, Professor A. A. Krishtal). The study included the following steps: isolation of total RNA from the primary material (blood serum); isolation of DNA from the primary material (blood serum); reverse transcription; polymerase chain reaction (PCR); restriction analysis; polymerase-chain reaction in real time; calculation of the number of amphilic molecules and construction of the calibration curve.

Statistical analysis of the results was carried out using the program "STATISTICA 6.1" (№ AGAR909E415822FA). Depending on the test results, parametric and nonparametric methods of statistics were used. Differences between the signs were considered statistically significant at $p<0.05$.

RESULTS

The average age of children who received standard therapy in combination with ursodeoxycholic acid was 7.84±0.39 years, without ursodeoxycholic acid – 8.90±0.52 years, practically healthy children – 8.15±0.60 years ($p>0.05$). No statistically significant differences were found in the study groups by age and gender ($p>0.05$). In the majority of the examined children (57.1%), clinical signs of functional disorders of the gallbladder and Oddi's sphincter were repeated. The duration of the disease in patients of the first and second groups ranged from 1 to 5 years.

During the study, family aggregation of biliary tract pathology in children's pedigrees with functional disorders of the gallbladder and Oddi's sphincter was determined. The burdened hereditary history of functional disorders of the gallbladder and Oddi's sphincter, chronic cholecystitis and cholelithiasis was found in 63 children (90%) of the first and second groups of the study.

Contributing factors to the onset or exacerbation of the disease in patients with functional disorders of the gallbladder and Oddi's sphincter were eating disorders (in 36 (72%) children of the main group and in 16 (80%) patients of the comparison group), psychoemotional load (25 (50%) and 8 (40%), respectively), hypodynamic lifestyle (15 (30%) and 25 (50%), respectively) and the presence of foci of chronic infection (7 (14%) and 4 (20%), respectively) ($p>0.05$).

All patients of both groups of observation were admitted to the hospital with manifestations of abdominal pain, dyspeptic and asthenovegetative syndromes of different duration and severity. 28 (56%) children of the first group and 13 (65%) patients of the second group complained of recurrent pain in the epigastrium and right hypochondrium, 13 (26%) children of the 1st group and 3 (15%) examined 2nd group – for recurrent pain in the umbilical region, 9 (18%) patients of the main group and 4 (20%) children of the comparison group – for pain in the right hypochondrium at the beginning of the observation ($p>0.05$). Pain was observed in the right hypochondrium in 42 (84%) children of the first group and in 18 (90%) patients of the second group on palpation of the abdomen ($p>0.05$). Dyspeptic symptoms were presented with nausea (22 (44%) patients of the main group and 8 (40%) of the comparison group), loss of appetite (26 (52%) and 9 (45%) respectively), belching bitter (11 (22%) and 4 (20%), respectively) before treatment ($p>0.05$). 34 (68%) patients of the 1st group and 14 (70%) children of the 2nd group had increased fatigue, decreased performance, and general weakness before treatment ($p>0.05$).

Significant differences in the regression of the main symptoms of abdominal pain, dyspeptic and astenovegetative syndromes in children of the main group and the comparison group were established after the treatment. Thus, symptoms of pain remained after treatment in 7 (14%) children of the first group and in 8 (40%) patients of the second group ($p<0.05$). All children of the main group after treatment with UDCA disappeared signs of dyspeptic syndrome. In 3 (15%) patients of the comparison group, after treatment without UDCA, complaints of nausea and decreased appetite persisted. In 9 (18%) patients of the 1st group and in 11 (55%) children of the 2nd group symptoms of the asthenovegetative syndrome were observed after the treatment ($p<0.05$).

Table 1. Results of the biochemical studies of blood serum in children with functional disorders of the gallbladder and Oddi's sphincter before and after treatment (M±m)

Children's group		Blood biochemical parameters		
		Alkaline phosphatase, n/l	Aspartate aminotransferase, n/l	Alanine aminotransferase, n/l
Children who received UDCA (n=50)	Before treatment	507,92±19,94 *	34,96±1,31 *	18,76±0,91 *
	After treatment	359,53±16,59 * **	26,18±0,97 **	14,96±0,64 **
Children who did not receive UDCA (n=20)	Before treatment	542,85±40,95 *	28,20±1,31 *	17,35±1,52
	After treatment	499,50±40,16 *	29,65±1,66 *	15,90±1,22
Practically healthy children (n=20)		202,95±9,07	24,20±0,89	14,50±0,57

Notes: * – p<0.05 – significantly compared with the parameter value in healthy children;
** – p<0.05 – significantly compared with the parameter value before treatmen.

Table 2. Results of the study of level serum expression of microRNA in children with functional disorders of the gallbladder and Oddi's sphincter before and after treatment (M±m)

Children's group		Level expression of microRNA		
		MicroRNA-378f, SU	MicroRNA-4311, SU	Micro-RNA-4714-3p, SU
Children who received UDCA (n=50)	Before treatment	2,02±0,57	1,48±0,35	5,56±1,50*
	After treatment	5,23±0,70 **	1,17±0,18	1,93±0,58* **
Children who did not receive UDCA (n=20)	Before treatment	2,08±0,96	1,58±0,47	4,66±1,57*
	After treatment	4,49±0,79	1,85±0,88	1,14±0,53* **
Practically healthy children (n=20)		5,05±2,85	3,07±1,41	53,62±10,42

Notes: * – p<0.05 – significantly compared with the parameter value in healthy children;
** – p<0.05 – significantly compared with the parameter value before treatmen.

The cholestasis markers of the biochemical hepatogram (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase) in patients with functional disorders of the gallbladder and Oddi's sphincter before treatment were significantly higher than those in healthy children (p>0.05) (table 1).

A significant decrease in the levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase in children with functional disorders of the gallbladder and Oddi's sphincter was found when analyzing the dynamics of biochemical hepatogram indicators after complex therapy using ursodeoxycholic acid. In children, who did not receive UDCA therapy, the above indicators did not have significant changes.

A significant decrease in the dynamic contractility of the gallbladder found in patients with functional disorders of the gallbladder and Oddi's sphincter both groups when studying the data of transabdominal dynamic ultrasound before therapy. The contractility of the gallbladder was 23.44±1.56% in children of the 1st group before treatment and 23.75 ± 2.29% in children of the 2nd group (p>0.05). The indicators of both groups were significantly lower than in practically healthy children – 49.55±1.11% (p<0.05).

The contractility of the gallbladder significantly increased to 45.56±1.68% compared in patients of the first group with the pre-treatment index (p<0.05). The contractility of the gallbladder averaged 29.69 ± 2.99% in children of the second group after treatment without UDCA and did not have a significant difference from the indicator before therapy (p>0.05).

The results of molecular genetic studies of serum expression of micro-RNA before and after treatment in children with functional disorders of the gallbladder and Oddi's sphincter and in healthy children are presented in table 2.

It was found that the parameters of the main group and the comparison group did not differ statistically significantly (p>0.05) during studying of the level of expression of microRNA-378f, microRNA-4311, microRNA-4714-3p in serum before treatment in children with functional disorders of the gallbladder and Oddi's sphincter.

It was determined that the activity profile of microRNA-4714-3p in the examined groups 1st and 2nd with functional disorders of the gallbladder and Oddi's sphincter was significantly lower than in healthy children in the analysis of the results of molecular genetic research (p<0.05).

However, there was no statistically significant difference

with the indicators in practically healthy children in the study of the level of expression of microRNA-378f and microRNA-4311 in children with functional disorders of the gallbladder and Oddi's sphincter before treatment (p>0.05).

Correlation analysis showed that the level of expression of microRNA-4714-3p in serum before therapy in the examined children positively correlated with a decrease in the contractile function of the gallbladder according to ultrasound examination (r=+0.36; p<0,05).

During the investigation it was established that the dynamics of the activity profiles of microRNA-378f and microRNA-4714-3p differed significantly after the therapy (table 1). After standard therapy in combination with ursodeoxycholic acid in children with functional disorders of the gallbladder and Oddi's sphincter, the level of expression of microRNA-378f approached the value of 5.23±0.70 SU, which was significantly higher than before therapy in children of this group (p<0.05). At the same time, after standard therapy alone without ursodeoxycholic acid, the level of microRNA-378f expression in children of the comparison group did not differ significantly from the pre-treatment index (p>0.05).

Against the background of standard therapy with the addition of ursodeoxycholic acid and without it, the expression profile of microRNA-4714-3p in serum of children with functional disorders of the gallbladder and Oddi's sphincter of the 1st and 2nd groups significantly decreased to the level of 1.93±0.58 SU and 1.14±0.53 SU respectively (p<0.05).

DISCUSSION

When studying the results of the study, positive clinical dynamics of manifestations of functional disorders of the gallbladder and Oddi's sphincter was determined after complex therapy with the use of ursodeoxycholic acid. This fact can be explained by an improvement in the rheological properties of bile and a decrease in the concentration of primary and secondary bile acids under the influence of ursodeoxycholic acid.

As a result of the study, it was found that at the beginning of observation of patients with functional disorders of the gallbladder and Oddi's sphincter, the average levels of expression of microRNA-378f, microRNA-4311, microRNA-4714-3p in serum of children of the main group and the comparison group were identical. It can be assumed that the significant decrease in the expression profile of microRNA-4714-3p before treatment in children of both groups was associated with the decrease in the contractile function of the gallbladder in children with functional disorders of the gallbladder and Oddi's sphincter.

Analysis of the study data showed that after complex therapy with and without ursodeoxycholic acid in children with functional disorders of the gallbladder and Oddi's sphincter, the dynamics of levels of expression of microRNA-378f and microRNA-4714-3p differed significantly. Probably, the significant increase in the expression profile of microRNA-378f, which was observed after the course of treatment with ursodeoxycholic acid, contributed to the restoration of bile drainage. It is possible that the decrease in the expression of microRNA4714-3p, which was observed after the course of treatment regardless of the use of ursodeoxycholic acid, may be due to modification of the diet.

CONCLUSIONS

1. As the result of studies it was found that in children with functional disorders of the gallbladder and Oddi's sphincter, changes in the profile of micro-RNA expression are observed.
2. These molecular genetic studies indicate the unique property of ursodeoxycholic acid in children with functional disorders of the gallbladder and Oddi's sphincter to influence the activity of the generation of gene regulators of cellular mechanisms of microRNA-378f and microRNA-4714-3p.
3. The use of ursodeoxycholic acid in the treatment of functional disorders of the gallbladder and Oddi's sphincter in children is justified due to its high efficiency.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

THE EFFECTIVENESS OF COMBINATED ANTIHYPERTENSIVE TREATMENT IN PATIENTS WITH ESSENTIAL HYPERTENSION OF THE II-ND STAGE DEPENDING ON THE TYPE OF DAILY BLOOD PRESSURE PROFILE AND THE TYPE OF REMODELLING OF THE LEFT VENTRICLE

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ABSTRACT

The aim: To evaluate the dynamics of structural and functional parameters of the heart, vessels in patients with hypertension of the II-nd stage under the influence of combined antihypertensive treatment depending on the type of the left ventricle remodelling and the profile of blood pressure (BP).

Materials and methods: The study involved 110 patients with hypertension of the II-nd stage. The ambulatory blood pressure monitoring, echocardiography and doppler examination of the shoulder arteries were performed. Initial treatment included bisoprolol, lisinopril, hydrochlorothiazide. With impossibility of BP lowering to the target level, amlodipine has been added.

Results: higher levels of BP in the "non-dipper" patients have led to the development of more pronounced changes in the heart and vessels compared with "dipper" patients. Three-component therapy was effective in patients with lower BP. The prescription of amlodipine has been found to be necessary for the majority of "non-dipper" patients and for the minority of "dipper". Combined therapy effectively controlled the BP at the level of the target and contributed to a decrease in the displays of remodelling of the heart and blood vessels.

Conclusions: combined therapy used for 6 months reduces displays of disadaptive heart and vascular remodelling, diastolic, endothelial dysfunction. With the lack of efficacy of the therapy, which includes bisoprolol, lisinopril, hydrochlorothiazide, the adding of amlodipine to it can reach the target level of BP

KEY WORDS: arterial hypertension, antihypertensive therapy, diastolic dysfunction, endothelial dysfunction

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INTRODUCTION

Nowadays arterial hypertension (AH) is one of the most important risk factors for the development of cardiovascular complications and mortality in the world, as it has become a medical and social problem. In 2016, about 12 million patients with AH were registered in Ukraine. Among the illnesses of the cardiovascular system (CVS) in the adult population, this indicator has reached 46.8%. High prevalence, disabling and fatal consequences of the illness require the search for ways to maximise the coverage of effective treatment of this population. Despite the availability of powerful opportunities for medical correction of high BP, the results of international and domestic studies indicate an inadequate level of its control. In modern strategic positions on the prevention and treatment of hypertension the emphasize the need to achieve the target level of BP, that reduces the risk of complications. Only 20% of the Ukrainians have a level that corresponds to the target. Increased BP affects the CVS, because excessive prolonged pressure on the target organs leads to pathological remodelling of the heart and blood vessels. This, first of all, is displayed by the left ventricular hypertrophy (LVH), the intima-media thickness (IMT) with subsequent functional deficiency of CVS, characterised by the development of diastolic dysfunction (DD) and endothelial dysfunction (ED). It has been defined, that LVDD is independent of the level of AP by the predictor of the prognosis worsening in patients with hypertension. The development of AH is a consequence of interaction of complex mechanisms, among which the leading role is played by ED, which is displayed in a violation of the ratio of factors of vasoconstriction and vasodilation. An important causal-consequential relationship between ED and the development and progression of AH has been proved in numerous studies. Increased BP is a consequence of various mechanisms responsible for its regulation (humoral, neuroendocrine systems, peripheral resistance, cardiac output, etc.). In this regard, the use of combined antihypertensive treatment, which acts on different parts of the AH pathogenesis, is the most effective [1, 2, 3, 4]. When prescribing combinative therapy for BP correction, the most effective therapy is the combination of the drug

acting on renin-angiotensin-aldosterone, sympathoadrenal systems, blocker of calcium channels (BCC), and thiazide diuretic [5, 6].

THE AIM

To evaluate the dynamics of structural and functional parameters of the heart, vessels in patients with hypertension of the II-nd stage under the influence of combined antihypertensive treatment depending on the type of the left ventricle remodelling and the profile of BP.

MATERIALS AND METHODS

The study involved 110 patients (70 women, 40 men) with hypertension of the II-nd stage, hypertensive heart, chronic heart failure (HHF) of the 0-I stage (average age 54.19 ± 0.89 years). The study have not included patients with symptomatic hypertension, diabetes mellitus, coronary heart disease. The 24-h mean, daytime mean, night-time mean systolic and diastolic BP (24-h SBPm, 24-h dBPm, dSBPm, dDBPm, nSBPm, nDBPm), the level of night-time SBP, DBP dipping (ndSBP, ndDBP), index of time of 24-h, day, night systolic and diastolic BP (TI 24-h SBP, TI 24-h DBP, TI dSBP, TI dDBP, TI nSBP, TI nDBP), magnitude and speed of morning increase of systolic and diastolic BP (MMI SBP, MMI DBP, SMI SBP, SMI DBP) have been defined. With echocardiography-study the end diastolic and systolic size (EDS, ESS), the volumes (EDV, ESV) of the left ventricle (LV), the size of left atrium (LA), the right ventricle have been defined with subsequent indexation of indices to the body area, the thickness of the posterior wall of the LV (LVPWTd), of the interventricular septum (IVSTd) in diastole, fraction of ejection (EF), relative thickness of the myocardium, index of mass of myocardium of LV (LVMMi). To characterise the diastolic function of LV (LVDF) the maximum speed of early (E) and late (A) diastolic filling has been measured. The E/A (diastolic index) has been defined, Te, Ta (time of early and late diastolic filling), Ei, Ai (area under the curve of early and late diastolic filling), % ΔA (percentage of LA input into the LV filling), Ta/Te (ratio of time of late versus time of early diastolic filling). The function of the endothelium of the vessels was determined by the method of Doppler examination of right shoulder artery using the Celermajer-Sorensen technique (endothelium-dependent vasodilatation – EDVD): the initial diameter of the artery was measured as well as the after reaction of hyperemia in 90 seconds. For determination of endothelial-independent vasodilatation (EIVD), the diameter of the artery was measured in 5 minutes after taking of 0,5 mg of nitroglycerine sublingually. The increase in diameter of this artery for more than 10% after the reaction of hyperemia after 90 seconds and more than 20% after 5 minutes after taking of nitroglycerin was considered normal. The intima-media thickness (IMT) of the same artery was also measured.

Initial treatment began with three-component therapy, which included the beta-blocker (BB) bisoprolol-hemifumarate at a dose of 5 mg per day, an angiotensin-converting enzyme inhibitor (ACEi) lysinopril-dihydrate in a dose of 10 mg per day, a diuretic hydrochlorothiazide at a dose of 12.5 mg per day. The duration of the initial treatment ranged from 5 to 10 days. The evaluation of the effectiveness of the prescribed therapy was carried out every 5-10 days. With insufficient antihypertensive effect of starting therapy, the dose of lisinopril was increased to 20 mg per day. If it was not possible to reach the target level for the above-mentioned three-component therapy, a calcium channel blocker (CCB) amlodipine in a dose of 5 mg per day was added to it. With its insufficient effectiveness, the dose of amlodipine was increased to 10 mg per day. Subsequently, after reaching the target BP level, if necessary, the dose of amlodipine was reduced from 10 to 5 mg per day. Evaluation of effectiveness of the prescribed treatment was carried out according to clinical data and data of ABPM after 3 months. With clinical benefit and the consent of the patient for further participation in the study, the prescribed therapy was continued for up to 6 months.

RESULTS

During ABPM, 2 types of daily BP profile have been identified: "dipper" (67 patients) and "non-dipper" (43 patients). According to the type of geometric models of LV, all patients were divided into 2 groups: a concentric hypertrophy of LV (CHLV) – 95 patients and an eccentric hypertrophy of LV (EHLV) – 15 patients. In the group of patients with CHLVB 58 patients had a "dipper" daily profile of BP, 37 – "non-dipper", in the group of patients with EHLV – 9 and 6 respectively.

In patients with CHLV, the three-component therapy was effective in 49 "dipper" patients and only in 3 "non-dipper" patients; respectively 9 and 34 patients with these profiles needed an additional amlodipine treatment. In patients with EHLV, the three-component therapy was effective in 5 "dipper" patients. Amlodipine was added to 4 patients for the "dipper" and to all "non-dipper" patients.

In patients with CHLV and "dipper" profile and effective response to the three-component therapy, a significant decrease in BP has been noted after 3 months, and subsequent stabilisation after 6 months (24-h SBPm from 147,88±2,07 to 130,53±2,19 and 129,37±1,89 mm Hg , 24-h dBPm from 92,10±1,83 to 80,29±2,39 and 79,51±2,21 mm Hg, dSBPm from 154,67±1,86 to 134,51±1,80 and 133,55±1,34 mm Hg, dDBPm from 96,04±1,74 to 82,63±1,83 and 82,06±1,71 mm Hg, nSBPm from 132,69±1,95 to 115,65±1,76 and 114,20±1,38 mm Hg, nDBPm from 82,24±1,63 to 70,57±1,73 and 70,00±1,59 mm Hg) ($p<0,001$). Accordingly, after 3 months significant decrease of indicators of pressure on target organs was noted, namely, 24-h (from 67,73±2,33 to 31,31±1,99 %, from 48,90±2,15 to 24,00±1,53 %), day (from 69,61±2,19 to 33,84±1,28 %, from 52,69±2,14 to 26,10±1,39 %), night (from 62,04±2,43 to 32,69±1,40, from 42,16±1,93 to 22,96±1,68 %) ($p<0,001$) time indexes of SBP, DBP with subsequent stabilization of these indicators in 6 months ($p <0.001$).

In patients with CHLV and "non-dipper" profile and effective response to the three-component therapy, a

similar dynamics of these indicators has been noted: decrease of 24-h SBPm from 159,33±0,58 to 132,00±1,73 and 130,67±0,58 mm Hg, 24-h dBPm from 97,33±1,15 to 82,67±0,58 and 82,33±1,15 mm Hg, dSBPm from 162,33±0,58 to 134,67±1,53 and 134,00±1,00 mm Hg, dDBPm from 99,67±2,08 to 83,67±0,58 and 83,00±0,00 mm Hg, nSBPm from 151,67±0,58 to 120,33±1,15 and 117,33±0,58 mm Hg, nDBPm from 92,00±2,00 to 74,00±1,00 and 71,67±0,58 mm Hg) (p<0,05). This contributed to a significant decrease in 3 months of the corresponding 24-h (from 81,33±1,53 to 33,00±1,00 %, from 67,00±2,65 to 25,33±1,15 %), day (from 82,33±1,15 to 35,33±1,15 %, from 69,69±2,14 to 27,67±1,53 %), night (from 80,67±1,15 to 32,67±0,58, from 69,67±3,06 to 24,33±1,15 %) (p<0,01) time indexes of SBP and DBP with the stabilization of these indicators in 6 months.

In the course of study of a group of patients with EHLV and the four-component therapy up to 3 months, the patients with "dipper" daily profile have left and only "non-dipper" patients have remained. In patients with EGLV and "dipper" profile at the background of three-component therapy, there was a significant stable decrease in the level of SBP and DBP after both 3 and 6 months (24-h SBPm from 148,40±3,44 to 131,00±2,92 and 130,50±1,73 mm Hg, 24-h dBPm from 91,80±1,79 to 78,60±0,55 and 77,75±0,96 mm Hg, dSBPm from 154,40±2,88 to 134,80±1,10 and 134,00±0,82 mm Hg, dDBPm from 96,00±1,22 to 83,00±1,87 and 83,00±2,16 mm Hg, nSBPm from 135,00±7,42 to 116,40±2,19 and 115,25±1,89 mm Hg, nDBPm from 83,40±4,28 to 71,20±2,28 and 71,25±1,89 mm Hg, (p<0,05)). This was accompanied by a significant decrease in 3 months of the 24-h (from 73,60±5,68 to 33,17±1,17 %, from 57,80±3,74 to 24,33±1,03 %), day (from 75,50±6,22 to 35,33±1,03 %, from 60,10±8,12 to 35,33±1,03 %), night (from 60,10±8,12 to 27,00±0,63, from 70,20±3,38 to 34,00±1,26 %) (p<0,05) time indexes of SBP and DBP with stabilization of these indicators in 6 months.

In the group of patients with CHLV and "dipper" profile whith ineffective responce to the three-component therapy, the prescription of amlodipine has allowed to lower BP to the target level, which was accompanied by a significant decrease in BP after both 3 and 6 months (24-h SBPm from 149,78±1,39 to 132,22±3,49 and 130,33±2,18 mm Hg, 24-h dBPm from 93,33±1,50 to 79,22±2,91 and 78,78±2,33 mm Hg, dSBPm from 156,22±0,97 to 135,33±1,32 and 134,00±1,58 mm Hg, dDBPm from 97,22±1,99 to 82,44±2,19 and 82,00±2,35 mm Hg, nSBPm from 134,89±1,27 to 116,44±1,67 and 115,33±1,12 mm Hg, nDBPm from 83,33±1,80 to 70,56±2,01 and 70,22±1,92 mm Hg) (p<0,01). Accordingly, after 3 months. significant decrease of indicators of pressure on the target organs has been noted, namely, 24-h (from 69,89±1,62 to 31,56±1,33 %, from 50,44±1,67 to 23,67±1,50 %), day (from 71,56±1,01 to 33,56±1,33 %, from 53,44±1,24 to 25,89±1,69 %), night (from 62,89±0,93 to 33,56±1,88, from 44,22±1,86 to 22,56±1,94 %) (p<0,001) time indexes of SBP and DBP with stabilization of these indicators in 6 months (p<0,001).

The group of patients with CHLV and "non-dipper" profile was marked with similar dynamics: decrease of 24-h SBPm from 160,24±1,72 to 133,27±2,35 and 131,24±1,57 mm Hg, 24-h dBPm from 98,68±1,61 to 81,60±1,98 and 80,62±1,78 mm Hg, dSBPm from 163,65±1,41 to 135,47±1,93 and 134,10±0,98 mm Hg, dDBPm from 101,76±1,72 to 83,70±1,73 and 82,93±1,28 mm Hg, nSBPm from 152,56±1,83 to 120,97±1,59 and 117,10±0,90 mm Hg, nDBPm from 94,09±1,60 to 73,73±1,62 and 71,86±1,16 mm Hg) (p<0,001). This contributed to a significant decrease in 3 months of corresponding 24-h (from 82,32±2,21 to 33,57±1,28 %, from 69,21±1,93 to 24,80±1,30 %), day (from 83,41±1,44 to 35,50±1,38 %, from 71,00±1,78 to 27,23±1,30 %), night (from 82,65±2,03 to 33,80±1,27, from 71,76±2,02 to 23,67±1,60 %) (p<0,001) time indexes of SBP and DBP with stabilization of these indicators in 6 months (p<0,001).

In patients with EHLV at the background of four-component therapy, a steady decrease in the level of SAP and DAP after both 3 and 6 month has been observed (24-h SBPm from 151,70±3,65 to 132,17±2,86 and 130,50±2,07 mm Hg, 24-h dBPm from 93,70±1,77 to 81,83±1,47 and 81,33±1,75 mm Hg, dSBPm from 157,00±2,91 to 135,83±1,47 and 133,83±0,98 mm Hg, dDBPm from 97,10±1,85 to 83,33±0,82 and 83,17±1,17 mm Hg, nSBPm from 140,40±3,04 to 118,50±2,95 and 116,17±1,47 mm Hg, nDBPm from 86,50±4,47 to 72,33±1,63 and 72,00±1,47 mm Hg, (p<0,05). This contributed to a significant decrease in 3 months of corresponding 24-h (from 68,80±4,71 to 31,60±1,67 %, from 52,60±4,30 to 23,40±0,89 %), day (from 71,20±5,54 to 33,80±1,30 %, from 54,80±6,91 to 26,00±0,73 %), night (from 64,00±7,92 to 32,20±1,92, from 47,00±8,79 to 22,60±0,55 %) (p<0,05) time indexes of SBP and DBP with stabilization of these indicators in 6 months.

It has been noted that in patients who received amlodipine, the average BP was higher than in patients with initially effective three-component therapy. Addition of amlodipine has allowed not only to lower the AP to the target level, but also to stabilise it throughout the observation period and to normalize the BP profile in the group of patients with its insufficient night decrease. Having compared both treatments in 6 months, a slightly higher antihypertensive effect has been observed at the background of four-component therapy in all groups of patients, since the percentage of lowering of BP average parameters compared with baseline data was slightly higher in patients receiving amlodipine. However, this difference was insignificant. The results of other studies confirm the fact that most patients require namely the combination of antihypertensive treatment. Therapy with using of ACEi, CCB, BB, diuretic for 6 months positively affects the parameters of brachial BP, reduces the degree of LVH and displays of DD [7, 8].

In evaluating of parameters of echocardiography in patients with CHLV and certain types of daily profile of BP at the background of three-component therapy, the significant improvement of structural-geometric parameters in both groups of patients was noted only in 6 months. However, more pronounced changes were observed in patients with "dipper" group: decrease of EDSi from 2,84±0,06

to 2,59±0,02 cm/m^2, ESSi from 1,90±0,04 to 1,74±0,01 cm/m^2, EDVi from 69,99±3,03 to 56,56±1,76 ml/m^2, LVMMi from 109,62±2,73 to 92,39±1,89 g/m^2 (p<0,001), LAi from 2,17±0,03 to 2,02±0,03 cm/m^2 (p<0,01), ESVi from 27,29±1,91 to 22,05±0,86 ml/m^2 (p<0,01), LVPWTd from 1,25±0,02 to 1,18±0,02 cm (p<0,01), IVSTd from 1,29±0,02 to 1,20±0,02 cm (p<0,01). In "non-dipper" group only the following parameters have changed: LAi from 2,20±0,02 to 2,06±0,02 cm/m^2, LVMMi from 112,93±0,91 to 97,43±1,62 g/m^2, ESVi from 29,87±0,99 to 23,79±0,86 ml/m^2, IVSTd from 1,35±0,02 to 1,25±0,01 cm (p<0,05). The evaluation of the parameters of DF has showed that in both groups of patients under the influence of three-component therapy a tendency to its improvement has been observed in 6 months, but this difference was insignificant.

Unlike the three-component, the four-component therapy contributed after 3 months to a minimal significant decrease of ESSi, ESVi, LVMMi (p<0,05) in the group of patients with CHLV and "dipper" profile and LAi, ESVi (p <0.05) in "non-dipper" group. In the evaluation of structural and geometric indices after 6 months, it has been noted that in both groups, the additional use of amlodipine contributed to their further improvement, moreover, more significant than at the background of three-component therapy. This was accompanied by a decrease of EDSi from 2,83±0,05 to 2,60±0,02 cm/m^2, LAi from 2,24±0,03 to 2,05±0,03 cm/m^2, LVPWTd from 1,28±0,02 to 1,20±0,01 cm (p<0,01), IVSTd from 1,33±0,02 to 1,22±0,01 cm (p<0,01), ESSi from 1,94±0,03 to 1,75±0,01 cm/m^2, EDVi from 71,77±1,03 to 58,80±0,90 ml/m^2, ESVi from 29,44±1,03 to 22,95±0,64 ml/m^2, LVMMi from 112,42±1,0 to 94,44±1,21 g/m^2 (p<0,001). In "non-dipper" group the decrease of EDSi from 2,80±0,06 to 2,62±0,02 cm/m^2, LVPWTd from 1,30±0,02 to 1,22±0,01 cm (p<0,01), IVSTd from 1,37±0,05 to 1,25±0,02 cm (p<0,05), LAi from 2,26±0,04 to 2,06±0,02 cm/m^2, ESSi from 1,95±0,03 to 1,76±0,01 cm/m^2, EDVi from 71,68±1,32 to 61,51±2,30 ml/m^2, ESVi from 30,51±1,00 to 23,80±0,90 ml/m^2, LVMMi from 114,66±1,82 to 97,73±1,56 g/m2 (p<0,001) has been observed.

It has been noted that addition of amlodipine has not only allowed to lower the AP to the target level, but also has contributed to more pronounced decrease in the degree of HLV, rigidity of the myocardium, and more significant improvement in the active relaxation of the lungs and intracardiac hemodynamics. Having evaluated functional indicators, it has been found out that unlike the three-component, the four-component therapy in both groups contributed to their improvement after 3 months with the following positive dynamics in 6 months. In "dipper" group the increase in the ratio E/A from 0,81±0,04 to 1,07±0,04 (p<0,01) has been noted, the decrease of %ΔA from 40,00±1,06 to 35,21±1,71 % (p<0,05) after 3 months. After 6 months E/A increased to 1,12±0,03 (p<0,001), %ΔA decreased to 35,41±0,83 %. Also the decrease of Tdec from 167,22±6,67 to 146,67±5,59 ms (p<0,001). In "non-dipper" group the correlation of E/A after 3 months decreased from 0,78±0,03 to 1,01±0,05 and to 1,06±0,04 after 6 months (p<0,001), %ΔA decreased from 42,07±1,32 to 36,97±1,74 % (p<0,05) and to 36,77±1,06 % (p<0,01), Tdec from 175,88±7,01 to 156,17±4,86 (p<0,05) and to 148,62±3,0 ms (p<0,01) respectively.

The evaluation of echocardiographic-study has showed that in patients with EHLV and certain types of daily profile, both treatment schemes have not caused after 3 months significant improvement of structural and geometric indicators. However, after 6 months in the group of patients with effective three-component therapy a significant decrease of EDSi from 2,86±0,04 to 2,64±0,03 cm/m^2 (p<0,05), ESSi from 2,06±0,04 to 1,81±0,02 cm/m^2 (p<0,05), EDVi from 82,25±2,28 to 68,28±1,19 ml/m^2 (p<0,001), ESVi from 38,37±1,89 to 28,14±0,37ml/m^2 (p<0,05), LVMMi from 106,94±2,74 to 93,68±1,10 g/m^2 (p<0,05) has been observed. The same dynamics in 6 months has been observed in the group of patients with the four-component therapy: decrease of EDSi from 2,93±0,06 to 2,63±0,04 cm/m^2 (p<0,05), ESSi from 2,11±0,05 to 1,81±0,02 cm/m^2 (p<0,01), EDVi from 83,88±2,01 to 65,98±1,64 ml/m^2, ESVi from 39,14±2,64 to 27,46±1,04 ml/m^2 (p<0,05), LVMMi from 106,74±1,42 to 93,13±1,10 g/m^2 (p<0,01).

In the assessment of LVDF, its improvement has been noted in both groups, which was characterised by an increase in E/A ratio from 0,81±0,02 to 1,02±0,01 m/s (p <0.01) in 3 months and up to 1,05 ± 0.00 m/s (p <0.001) in 6 months in "dipper" patients with under the influence of the three-component therapy and from 0,79 ± 0,02 to 1,02 ± 0,01 m/s (p <0.001) in 3 months and to 1,06 ± 0,01 m/s (p <0,001) in 6 months in 'non-dipper" patients under the influence of the four-component therapy.

In numerous studies, it has been shown that a decrease in LVMMi is associated with an improvement of DF, a decrease of LA size, and of vascular stiffness. This is essential because it is important not only to prescribe drugs that lower BP, but also to have a positive effect on myocardial hypertrophy and elastic properties of blood vessels. The combination of ACEi and CCB is exactly the same [9].

In assessing the dynamics of structural and functional parameters of vessels, the following results have been obtained. In patients with CHLV with "dipper" profile the significant reduction of the processes of pathological remodelling of the vascular wall was noted after 3 months, which was displayed by the decrease of the IMT from 0,45 ± 0,02 to 0,39 ± 0,01 mm (-13,33%) (p <0,01) and to 0,34 ± 0,01 mm (-24,44%) after 6 months. Having analyzed the function of the endothelium, it has been noted that 3 months treatment with bisoprolol, lisinopril, hydrochlorothiazide contributed to the increase in the growth of the diameter of the brachial artery after the reaction of hyperemia from 10,26 ± 0,18% to 21,36 ± 0,34% (p <0.001), followed by its increase to 23,81 ± 0,22% in 6 months (p <0.001). Having evaluated EIVD after 3 months, the increase in the growth of the diameter of the brachial artery from 19,39 ± 0,23% to 28,82 ± 0,38% (p<0.001) has been noted with the subsequent improvement of this indicator in 6 months (increase in growth to 30,04 ± 0,30% (p <0.001)).

In patients with CHLV and "non-dipper" profile the three-component therapy either after 3 months, or after 6 months have not contributed to a significant reduction of the IMT (from 0,57 ± 0,09 to 0,47 ± 0,03 (-17,54%) and to 0,40 ± 0,00 mm (-29,82%). However, in this group of patients, the minimal significant improvement of the endothelium function has been observed, which was characterised by an increase in the growth of the diameter of the brachial artery after the reaction of hyperemia after 3 months from 8,19±0,17% to 18,01 ± 0,78% and to 21,59±1,02% after 6 months ($p<0.05$), and after the test with nitroglycerin from 18,08 ± 0,53% to 24,14 ± 1,7% and 27,08 ± 0,93% respectively ($p <0.05$).

Adding of amlodipine to patients with CHLV with "dipper" profile has allowed to reduce of the IMT in this group after 3 months from 0,49 ± 0,03 to 0,41 ± 0,01 mm (-16,33%) ($p <0,05$) and to 0,39 ± 0,01 mm (-20,41%) in 6 months ($p <0.05$). EIVD has improved from 10,10 ± 0,30% to 19,11 ± 1,94% ($p<0.001$) after 3 months and to 23,18 ± 0,0,59% after 6 months ($p<0,001$). Unlike the three-component therapy, the four-component therapy in patients with CGLV and "non-dipper" profile has proved to be more effective, since under its influence already after 3 months a significant reduction in the thickness of CIM from 0,54 ± 0,02 to 0,46 ± 0,01 mm (-14,81%) ($p <0,01$) and after 6 months to 0.41 ± 0.01 (-24.07%) ($p <0.001$) has been onserved. The same positive dynamics has been noted in the evaluation of endothelial function, which was followed with improvement in 3 and 6 months both in EDVD (increase in the growth of the diameter of the brachial artery from 8,13 ± 0,19% to 18,99 ± 0,35% and up to 21,92 ± 0.33% ($p<0.001$), as well as in EIVD (increase in the growth from 16,87 ± 0,17% to 26,43 ± 0,27 and to 27,73 ± 0.21% ($p <0.001$)).

A similar tendency has been observed in patients with EHLV. Thus, in "dipper" patients the three-component therapy has contributed to a significant improvement of both EDVD (increase in the growth of the diameter of the brachial artery from 9,27 ± 0,6% to 19,73 ± 0.33% ($p<0.01$) in 3 months and up to 22,03 ± 0,24% in 6 months ($p <0,01$)) and in the EIVD (increase in the growth from 17,08 ± 0,59% to 24,10 ± 0,55% ($p<0,01$) and up to 26,29 ± 0,37% ($p <0,01$) respectively). At the background of the four-component therapy in "non-dipper" patients the improvement of EDVD has been noted from 9,47 ± 0,19% to 19,83 ± 0,63% after 3 months and up to 22,30 ± 0,58% in 6 months ($p <0,001$), EIVD from 17,14 ± 0,57% to 24,32 ± 0,60% ($p <0,001$) and up to 26,82 ± 0,24% ($p<0.001$) respectively. After 6 months the reduction of the IMT in both groups has been observed (under the influence of the three-component therapy from 0,46 ± 0,02 to 0,40 ± 0,00 mm (-13,04%), at the background of the four-component therapy from 0,45 ± 0,02 to 0,37 ± 0,02 mm (-17.78%), but this difference was unreliable. According to numerous studies, the violation of the elastic properties of the arteries, regardless of the level of BP, is associated with LVH, the IMT, LVDD. ACEi and CCB positively effect on the stiffness of the myocardium and arteries. The extent of the effect of ACEi on the arteries may be partially genetically determined.

DISCUSSION

Analysing the structural and functional parameters of the heart and blood vessels, it has been established that higher levels of BP and longer duration pressure on the target organs in "non-dipper" patients contributed to the development of a more pronounced LVH, thickening of IMT, increased rigidity of the myocardium and vessels, violation of their relaxation. Subsequently, this has led to more significant violations of LVDF and EF of vessels.

Evaluating the effectiveness of combined antihypertensive treatment, it has been found that three-component therapy was initially effective in patients with lower BP. An additional use of amlodipine to lower BP to the target levels has appeared to be necessary for the majority "non-dipper" patients and the minority of "dipper" patients. Both treatment schemes contributed to a significant decrease in BP after 3 months with subsequent stabilisation in 6 months. At the background of the three-component therapy after 6 months the reduction of displays of pathological remodelling, including LVH only in "dipper" patients has been noted. However, in both groups of patients, this treatment have not contributed to a significant improvement in DF even in 6 months. The additional use of amlodipine has not only allowed to lower the BP to the target level, but also significantly to reduced after 6 months the displays of pathological remodelling and DD in patients with both types of 24-h profile of BP.

It is known that ACEi improves the function of the endothelium by preventing the implementation of mitotic and proliferative effects of AT II, blocking the synthesis of peroxydanion, which inactivates NO, increases the formation of NO, slows the degradation of bradykinin, reduces the synthesis of collagen and the development of fibrosis. CCB limits the vasoconstrictive effect of AT II, endothelin-1 by reducing the intracellular calcium pool, possess antioxidant properties, stimulate the synthesis of NO. Thus, CCB provides additional organoprotective action to the ACEi. According to the literature, including the research of HAMLET, ELVERA, ROZALIA, the combination of lisinopril, amlodipine and lisinopril, amlodipine, hydrochlorothiazide has broad evidence base of clinical efficacy and safety, possess long proven antihypertensive effect, synergy component, allows to monitor the morning rise of BP, has confirmed organoprotective properties, ability to reduce the level of LVH, manifestations of DD, proteinuria, ED, is well tolerated by patients. It has been proved that this combination helps to reduce the risk of development of the primary combined endpoint of "death and cardiovascular complications" in patients with high-risk [10, 11].

Thus, the obtained by us results show the high efficiency of combined antihypertensive therapy, which includes ACEi, BB, diuretic, CCB. Both treatment schemes not only have had the pronounced antihypertensive effect, but also have reduced the displays of pathological remodelling of the CVS, which further reduces the risk of complications and mortality.

CONCLUSIONS

In patients with essential hypertension of the II-nd stage with hypertensive heart, HHF) of the 0-I stage with the daily "non-dipper" profile higher mean 24-h, day, and night SBP and DBP are regis-

tered, as well as the corresponding time indices, speed of morning increase of SBP and DBP than in patients with daily "dipper" profile, which leads to more pronounced structural-geometric and functional changes in the heart and blood vessels. Prolonged, during 6 months, combined antihypertensive therapy reduces the displays of disadaptive remodelling of the cardiovascular system, diastolic and endothelial dysfunction. With the lack of efficacy of the three-component antihypertensive therapy, which includes BB bisoprolol, ACEi lisinopril, diuretic hydrochlorothiazide, the addition of CCB amlodipine allows to reach the target level of BP, reduces the pressure burden on the target organs and reduces the risk of cardiovascular complications and mortality.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

FETAL ANATOMY OF PARATHYROID GLANDS

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ABSTRACT

The aim: To study the forms of anatomical variability of the external structure of the upper and lower parathyroid glands in the fetal period of human ontogenesis.

Materials and methods: The study involved 48 specimens of human fetuses with 81,0-375,0 mm of crown-rump length (CRL). The study was conducted by means of macro-microscopic preparation, morphometry and variation statistics method.

Results: The age and individual anatomical variability, complex way of development and formation of synotopic embryotropographic correlations of the upper and lower parathyroid glands in the prenatal period of human ontogenesis create numerous prerequisites for the emergence of variants of their external structure and topography in the fetuses of both different and the same age groups.

Conclusions: There is a significant anatomical variability of the upper and lower parathyroid glands in 4-10-month-old fetuses, which is manifested by varieties of their shape and topical location. Aplasia of the upper parathyroid glands, which was found in two human fetuses aged 7 months, was due to the fetures of their organogenesis and the formation of syntopy in the embryonic and prefetal periods of their development. Parathyroid glands are mainly supplied with blood by the branches of the inferior thyroid artery. The branches of the upper thyroid artery and the arteries of adjacent organs: larynx, trachea and esophagus are involved in the blood supply. The right and left inferior thyroid veins are tributaries of the corresponding brachiocephalic vein, paired (right and left) superior and middle thyroid veins are those for the internal jugular vein.

KEY WORDS: parathyroid glands, ontogenesis, anatomical variability

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INTRODUCTION

The problem of specific complications in thyroid surgery is very acute in both Ukraine and in the world, which is associated with a large number of operations on the thyroid gland (TG) (in Ukraine there are 11-12 thousand operations per year) and the incessant tendency to increase the incidence of diseases [1]. Among the main specific complications in thyroid surgery (damage to the laryngeal nerve with subsequent paresis of the larynx and hypoparathyroidism with hypocalcemia syndrome), it is the postoperative reduction of parathyroid hormone secretion that is the most common type of undesirable effects of surgery occurring in 20-60% of patients and which causes significant deterioration in the quality and duration of their life [2, 3, 4]. Therefore, the data on the features of the topographic anatomy of the parathyroid glands (PTG) and possible variants of their structure are important to avoid postoperative hypoparathyroidism [5, 6, 7].

The identification of PTG during surgical interventions may be complicated due to their small size, number, anatomical variations, close location to important structures and unreliable visualization [8, 9, 10].

The PTG play an important role in controlling the level of calcium and, therefore, have a direct impact on muscle contraction and neurotransmission. Structural variants and ectopic location of the PTG increase the complexity of surgical interventions on the TG and PTG [11, 12].

Heterotopia of the PTG due to aberrant migration in the early stages of development and the impossibility of their identification may lead to errors in surgical interventions on the TG and PTG, in the mediastinum and pericardium area. According to anatomical studies, the incidence of PTG ectopia is about 2-43% and 14-16% in patients with primary and secondary hyperparathyroidism, respectively. The ectopic localization of the lower PTG in the anterior mediastinum is most frequently observed, and the most common heterotopia of the upper PTG is the tracheo-esophageal groove or the retroesophageal region [13, 14].

Finding patterns of the development of the structure and topography of fetal organs and systems is important for the interpretation of the true direction of organogenesis processes, mechanisms of normal body growth, the emergence of anatomical variants and congenital malformations [15]. Studying the shapes of anatomical variability of organs and structures, in

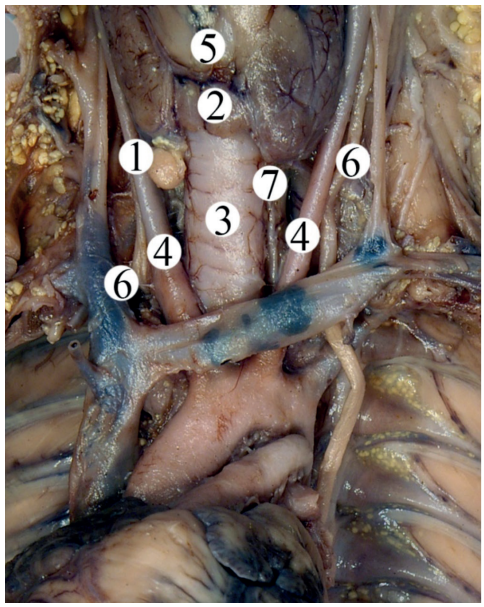

Fig. 1. The organs and structures of the neck and thoracic cavity of the fetus with 170.0 mm of CRL. Gross specimen. Augm. 2,6x:
1 – the right lower parathyroid gland;
2 – the isthmus of the thyroid gland;
3 – the trachea;
4 – the common carotid arteries;
5 – the arch of the cricoid cartilage;
6 – the vagus nerves;
7 – the left recurrent laryngeal nerve.

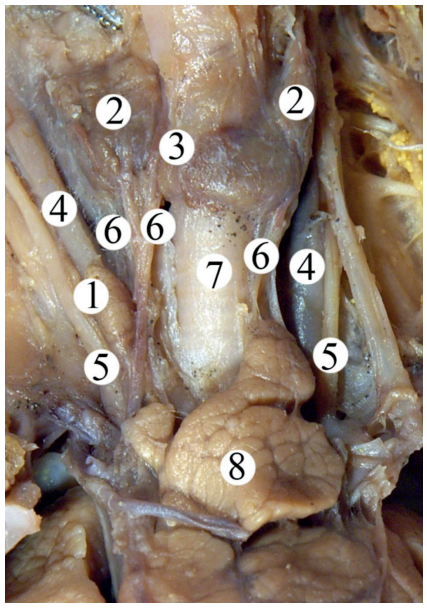

Fig. 2. The organs and structures of the neck and thoracic cavity of the fetus with 190.0 mm of CRL. Gross specimen. Augm. 2,7x:
1 – the right lower parathyroid gland;
2 – the thyroid gland lobes;
2 – the isthmus of the thyroid gland;
4 – the common carotid arteries;
5 – the vagus nerves;
6 – the inferior thyroid veins;
7 – the trachea;
8 – the thymus.

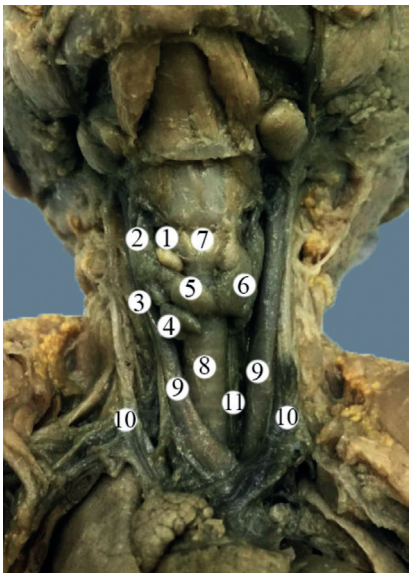

Fig. 3. The organs and structures of the neck and thoracic cavity of the fetus with 192,0 mm of CRL. Gross specimen. Augm. 2,3x:
1 – the right upper parathyroid gland;
2 – the right lateral lobe of the thyroid glandправа;
3 – the right middle lateral lobe of the thyroid gland;
4 – the right lower lateral lobe of the thyroid gland;
5– the right paramedian lobe of the thyroid gland;
6 – the left lobe of the thyroid gland;
7 – the arch of the cricoid cartilage;
8 – the trachea;
9 – the common carotid arteries;
10 – the internal jugular veins;
11 – the left recurrent laryngeal nerve.

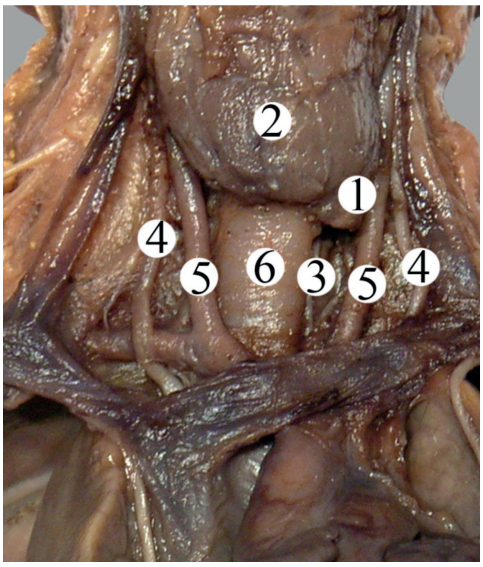

Fig. 4. The organs and structures of the neck and thoracic cavity of the fetus with 225,0 mm of CRL. Gross specimen. Augm. 2,1x:
1 – the left lower parathyroid gland;
2 – the thyroid gland;
3 – the left recurrent laryngeal nerve;
4 – the vagus nerves;
5 – the common carotid arteries;
6 – the trachea.

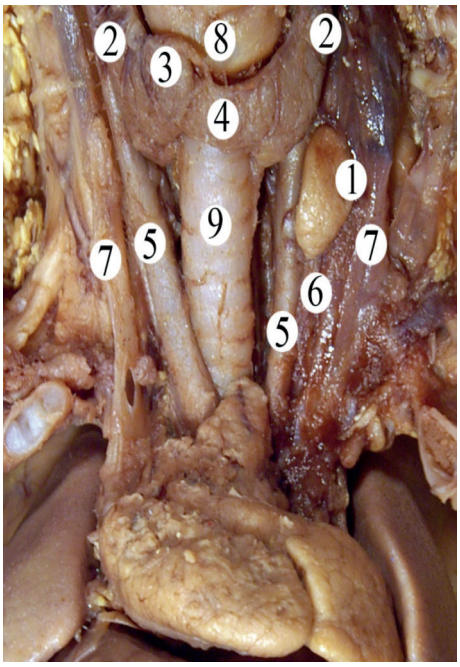

Fig. 5. The organs and structures of the neck and thoracic cavity of the fetus with 240,0 mm of CRL. Gross specimen. Augm. 1,8x:
1 – the left lower parathyroid gland;
2 – the lobes of the thyroid gland;
3 – the pyramidal process of the thyroid gland;
4 – the thyroid gland isthmus;
5 – the common carotid arteries;
6 – the left vagus nerve;
7 – the internal jugular veins;
8 – the cricoids cartilage arch;
9 – the trachea.

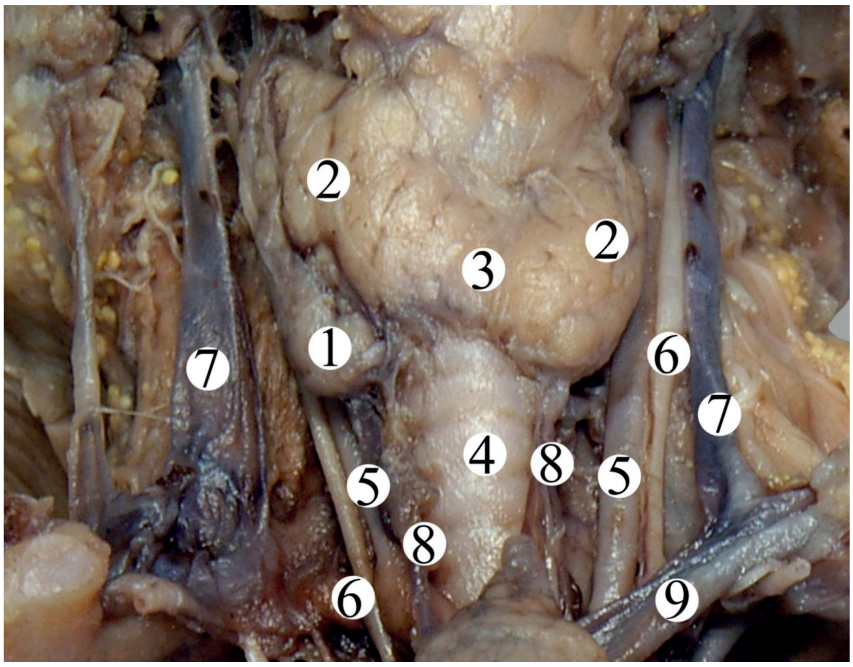

Fig. 6. The organs and structures of the neck and thoracic cavity of the fetus with 280,0 mm of CRL. Gross specimen. Augm. 2,4x:
1 – the right lower parathyroid gland;
2 – the lobes of the thyroid gland;
3 – the isthmus of the thyroid gland;
4 – the trachea;
5 – the common carotid arteries;
6 – the vagus nerves;
7 – the internal jugular veins;
8 – the lower thyroid veins;
9 – the left brachiocephalic vein.

particular the glands of the internal secretion during the fetal period of human ontogenesis is a comprehensive task. The sources of literature contain rare reports on prenatal morphogenesis and variant anatomy of the PTG [8, 16], which does not fully reflect their age and individual anatomical variability.

THE AIM

To study the forms of anatomical variability of the external structure of the upper and lower parathyroid glands in the fetal period of human ontogenesis.

MATERIALS AND METHODS

The study involved 48 specimens of human fetuses with 81,0-375,0 mm of crown-rump length (CRL) by using gross and fine dissection and morphometry. The study of fetal spesimens with a mass of 500.0 g or more was conducted in the Chernivtsi region Communal Medical Establishment «Pathologoanatomical Bureau» in accordance with the cooperation agreement. The study also involved spesimens of fetuses of all ages without external signs of anatomical abnormalities or abnormalities of the cervical and anterior thoracic areas from the Museum of the Department of Human Anatomy named after M.G. Turkevych of HSEI of Ukraine «Bukovinian State Medical University».

The Commission on Biomedical Ethics of Bukovinian State Medical University revealed no moral and legal violations during medical scientific research.

RESULTS AND DISCUSSION

At the beginning of the fetal period of human ontogenesis, PTG develop intensively. 11 of the examined 4-5-month-old fetuses had the variability of the shape of the right and left upper (UPTG) and lower (LPTG) parathyroid glands. For instance, the elongated shape of UPTG and its variants (elongated-oval, elongated-round, spindle-shaped) was observed in 10 cases (45.5%), oval shape in 7 observations (31.8%) and bean-shaped one in 5 cases (22, 7%). The following variants of the shape of LPTG were found: round – 8 observations (36.4%), oval – 6 cases (27.3%), elongated – 5 (22.7%) and crescent-like one- 3 (13.6%). The UPTG in 4-5 month old fetuses are mainly located at the level of the middle third of the posterior surface of the thyroid gland (9 cases, 40.9%) or between the upper and

middle third of the thyroid gland (6 observations, 27.3%), less frequently – at the level of the upper third of the TG lobes – 3 cases (13.6%), on the border of the middle and lower third of the thyroid gland – 2 observations (9.1%) or in the thickness of the thyroid gland – 2 (9.1%). The right and left LPTG take the following positions: at the level of the lower third of the posterior surface of the thyroid gland (8 cases, 36.4%), below the TG lobes – 6 (27.3%), on the border of the middle and lower third of the thyroid gland – 4 (18, 2%), within the limits of the vascular-nerve bundles of the neck – 3 (13.6%) and in the thickness of the thyroid gland – 1 case (4.5%).

In particular, the fetus with 170.0 mm CRL had an H-shaped thyroid gland and the right LPTG was located under its lower pole. The latter is rounded, 3.5 mm high and 4.5 mm wide, adjacent to the lower end of the right thyroid lobe and is located between the trachea and the right common carotid artery (Fig. 1). The left lobe of the thyroid gland is adjacent to the lateral surface of the trachea, cricoid cartilage, and the lateral surface of the esophagus. The isthmus of the thyroid gland is at the level of the 3rd tracheal cartilage. The topical placement of the right and left UPTG, as well as the left LPTG is without any variant features.

The thyroid gland of a fetus with 190.0 mm of CRL is characterized by a horseshoe shape. The right LPTG which is oval, 7.0 mm high and 3.5 mm wide, is located laterally and 8.0 mm below the basement of the right lobe of the thyroid gland. The back surface of the right side LPTG is closely adjacent to the right common carotid artery. The right vagus nerve is adjacent to the lateral surface of the LPTG, and the right lower thyroid veins to its paramedian surface. (Fig. 2).

Studying 21 fetuses aged 6-7 months in 2 observations (255.0 and 260.0 mm of CRL) found aplasia of the right and left UPTG. The fetus with 192.0 mm of CRL had the atypical asymmetric shape of the thyroid gland, which consisted of 5 lobes: the left one;the right paramedian; the right upper lateral one; the right middle lateral and the right lower lateral ones. The fetus did not have the isthmus of the thyroid gland or the pyramidal lobe either. The right UPTG, which is oval, 5.0 mm long and 3.5 mm wide, is located atypically, namely: medially of the right upper lateral lobe of the thyroid gland and slightly above the right paramedian lobe of the thyroid gland. The oblique part of the cricothyroid muscle is located medially to the right UPTG. The back surface of the right HPTG is adjacent to the first tracheal ring (Fig. 3).

6-7 month-old fetuses were found to have the following types of UPTG: oval – 13 cases (34.2%), elongated – 10 (26.3%), rounded – 7 (18.4%), crescent-shaped – 5 (13.2 %), lentil-shaped – 3 (7.9%). The LPTG is characterized by the variability of the shape as well: rounded – 16 (38.1%), lentil-shaped – 10 (23.8%), bean-shaped – 8 (19.1%), oval – 5 (11.9%), elongated – 3 (7.1%). In 6-7 month-old fetuses UPTG are generally localized between the upper and middle third of the posterior surface of the thyroid gland – 15 cases (39.5%) and at the level of the middle third of the thyroid gland – 11 (28.9%), less frequently – at the level of the upper third of the thyroid gland – 7 (18.4%) or within the vascular nerve bundles of the neck, more laterally to the thyroid gland lobes – 5 (13.2%). LPTG are topically found within the lower third of the thyroid back surface lobes – 19 observations (45.2%), below the thyroid gland lobes – 11 (26.2%), on the border of the middle and lower thirds of the thyroid gland lobes – 8 (19%), within the vascular-nerve bundles of the neck – 2 (4.8%) or behind the sternum – 2 (4.8%). The fetus with 225.0 mm CRL was found to have an atypical shape of the thyroid gland which looked like the letter «L», represented by a vertical (the right lobe with a pyramidal process) and a horizontal (the isthmus and the left lobe) parts, as well as the position of the left LPTG under the lower pole. The left LPTG is lentil-shaped, 3.0 mm high and 6.0 mm wide, adjacent to the lower edge of the left lobe of the thyroid gland and is located between the trachea and the left common carotid artery (Fig. 4). The anterior surface of the left LPTG is closely adjacent to the left recurrent laryngeal nerve.

The fetus with 240.0 mm of CRL was found to have heterotopia of the left LPTG located in the left neurovascular bundle of the neck, between the left common carotid artery and the left internal jugular vein and is 4.0 mm below the left lobe of the thyroid gland (Fig. 5). The LPTG is oval and its posterior surface adjoins the anterior surface of the left vagus nerve and the anterior-lateral surface of the left common carotid artery. The left LPTG is 11,0 mm long and – 5,5 mm wide. The TG, which is horseshoe-shaped, consists of the right and left lobes, which are located on the anterior surface of the trachea, at the level of I-III tracheal cartilages. A pyramidal process leaves the right lobe of the thyroid gland. It should be noted that the isthmus of the thyroid is below the arch of the cricoid cartilage, while the right lobe and the pyramidal process partly cover the arch of this cartilage. The right common carotid artery closely adjoins the right lobe of the thyroid gland laterally, while the left vagus nerve and the left internal jugular vein are adjacent to the left TG lobe. The thyroid cartilage consists of two quadrilateral, symmetrical plates, the right and the left ones, connected at an obtuse angle.

At the end of the fetal developmental period (16 fetuses aged 8-10 months were studied), there is a variability of the shape and position of the right and left UPTG and LPTG. The following variants of the UPTG shape were observed: oval (13 cases, 40.6%), rounded – 9 (28.1%), elongated – 7 (21.9%), flat – 2 (6.3%), droplet-shaped one – 1 (3.1%). The LPTG are more likely to be rounded – 17 observations (53.1%) and lentil-like ones – 10 (31.3%) ; rarely they are elongated – 4 (12.5%) and oval – 1 (3.1%). UPTG are, as a rule, located on the border of the upper and middle third of the posterior surface of the thyroid gland lobes- 12 cases (37.5%), or at the level of the upper third of the thyroid gland – 11 (34.4%), rarely – at the level of the middle third of the thyroid gland – 4 (12,5%), in the thickness of the thyroid gland – 3 (9,4%) or above the thyroid gland lobes – 2 (6,2%). LPTG are mainly localized at the level of the lower third of the posterior surface of the thyroid gland lobes – 15 observations (46.9%). One or

two-sided position of the LPTG under the lower ends of the TG lobes thyroid was found in 7 cases (21.9%), on the border of the middle and lower thirds of the thyroid gland lobes – 4 (12.5%), in the thickness of the thyroid gland – 4 (12.5%) and within the corresponding neurovascular bundle of the neck – in 2 observations (6.2%). It should be noted that sometimes one and the same fetus has an asymmetry of the shape of the right and left UPTG and LPTG.

The fetus with 280.0 mm of CRL was found to have an atypical position of the right LPTG – caudolaterally to the right lobe of the thyroid gland (Fig. 6). The latter is butterfly-shaped. The right LPTG, which is rounded, 5.0 mm high and 6.5 mm wide, is placed between the lateral wall of the trachea and the right vagus nerve. The posterior surface of the right LPTG is adjacent to the anterior semicircle of the right common carotid artery.

The blood supply of the PTG is mainly carried out by the branches of the inferior thyroid gland. The branches of the superior thyroid artery and the arteries of the adjacent organs: the larynx, the trachea and the esophagus are involved in the blood supply of the PTG. The thyroid venous plexus is located on the anterior surface of the cervical part of the trachea and thyroid gland, arches of the cricoid cartilage and the plates of the thyroid cartilage. The right and left inferior thyroid veins are tributaries of the corresponding brachiocephalic vein while the paired (right and left) superior and middle thyroid veins are those of the internal jugular vein.

CONCLUSIONS

1. The age and individual anatomical variability, a difficult way of development and formation of syntopic embryonic tropographic correlations of UPTG and LPTG in the prenatal period of human ontogenesis create numerous prerequisites for the emergence of variants of their external structure and topography in the fetuses of both different and the same age group.
2. 4-10 month-old fetuses are characterized by a significant anatomical variability of UPTG and LPTG which manifests itself by the varieties of their shapes and topical location.
3. The UPTG aplasia, which was found in two human fetuses aged 7 months, is due to the features of their organogenesis and the formation of syntopy in the embryonic and pre-fetal developmental periods.
4. The obtained data on the variant anatomy of the PTG should be taken into account by surgeons-endocrinologists and doctors of ultrasound diagnostics during diagnostic and operative manipulations.

Prospects of further research. The conducted study on the dynamics of the formation of the shape and size of the PTG in 4-10- month-old fetuses indicates the need to further elucidate their variant anatomy in newborns.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

SUBSTANTIATION AND DIFFERENTIAL APPROACH TO OPERATIVE TREATMENT OF PATIENTS WITH PSORIATIC ARTHRITIS

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ABSTRACT

The aim: To improve the psoriatic arthritis treatment outcomes by establishing and implementing into practice operative treatment.

Materials and methods: The study basis is represented with the results of examination and treatment of 252 patients with psoriatic arthritis. The biopsy samples from the problem psoriatic eruption and intact skins regions, received from 74 patients, were studied.

Results: The study has established presence of Staphylococcus aureus in affected skin regions. The authors present their classification of structural muscular-skeletal disorders associated with psoriatic arthritis, which will substantiate the volume and character of operative interventions.

Conclusions: Detection of numerous opportunistic pathogenic microorganism colonies stipulates for the necessary skin debridement while planning further operative treatment, aimed at correction of anatomical and biological abnormalities. The operative intervention volume depends on the severity of structural dysfunctions.

KEY WORDS: psoriatic arthritis, operative treatment

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INTRODUCTION

Psoriatic arthritis is an independent nosological type of non-specific general disorder of muscular-skeletal system associated with psoriasis. The disease has multifactorial genesis with hereditary predisposition and is characterized by chronic progressive recurring development which leads to continuous and permanent disability of the patients.

Psoriatic arthritis (PA) affects from 5% to 60% of patients with psoriasis (P), more often men. The disease may develop at any age, but it most often affects the working age population (from 30 to 50 years), so the question of prevention and treatment of muscular-skeletal dysfunctions associated with psoriasis, particularly at its early stage, is medically and socially urgent [1-5].

Such statistical difference may be explained by different places of residence, though, in our mind, it is related to some drawbacks of the condition diagnostics [6]

Despite considerable achievements in the disease etiology and pathogenesis study, many questions are still unclear. The basic causing factors of the disease are: heredity; immune diseases, disordered mineral metabolism and connective tissue metabolism, chronic infection foci, various diseases affecting other organs, psychoasthenic and neuropathic conditions, negative effect of the environment (temperature, radiation, etc.), injuries and traumas [7-10].

Within this context, PA should be considered as a process with multifactorial nature and pathological changes related to the additional action of hereditary and environmental factors [1].

Modern conservative treatment of psoriatic arthritis using the newest and traditional medications provides for significant improvement in the patients' condition [11-13].

Though, it is necessary to mention that with considerable structural and functional dysfunctions in any region of the muscular-skeletal system, the medication therapy effectiveness is minimum, or, to say directly, ineffective. There are single reports about operative treatment of such patients, based on several cases, which cannot evidence about the indications and contraindications to it [14].

So, the PA in modern orthopedics represents an urgent problem, as etiology and pathogenesis of this process haven't been defined completely as well as the methods of diagnostics, complex orthopedic prevention and treatment, which decreases the specialized treatment quality for such patients.

THE AIM

To improve the psoriatic arthritis treatment outcomes by establishing and implementing into practice operative treatment.

MATERIALS AND METHODS

The complex examination included clinical, general laboratory, immunological, biochemical, roentgenological, sonographical, densitometrical, arthroscopic and morphological study methods.

The study represents the results of examination and treatment of 252 patients with PA of various active stages, spread and severity. 82 patients have undergone operative treatment.

The authors have also studied 74 patients with progressing psoriasis vulgaris, moderate or severe dermatosis. The

Table I. Groups of patients by age, age period of disease onset and disease duration.

Age of the patients (years old)	Amount	%	Age period of the disease onset			Duration of the disease		
			Years old	amount	%	years	amount	%
21 – 30	17	6.7	Less than 20	25	10.0	Less than 10	123	48.8
31 – 40	42	16.6	21-30	59	23.3	11 – 20	92	36.6
41 – 50	67	26.7	31-40	75	30.0	21 – 30	17	6.7
51 – 60	67	26.7	41-50	59	23.3	31 – 40	17	6.7
61 and more	59	23.3	51-60	34	13.4	41- 50	3	1.2
Totally	252	100		252	100		252	100

Table II. Incidence of the muscular-skeletal disorders related to localization

Localization	Incidence, %
Shoulder joints	12. 6
Elbow joints	27. 2
Radial wrist joints	10. 2
Digital hand joints	74. 7
Кульшові суглоби	13. 4
Iliosacral joints	53. 2
Pelvic joint	58. 2
Ankle foot joint	29. 7
Metatarsophalangeal and interphalangeal foot joints	64. 7
Psoriatic spondyloarthritis	21. 6
Pubic symphysis	14.2

Table III. Structural dysfunctions of the muscular-skeletal system with associated with psoriatic arthritis

Roentgenological signs	Stage			
	I	II	III	IV
Increased rate and loss of the periarticular tissue structure	+	+ +	+ +	+ + +
Osteoporosis	+	+ +	+ + +	+ + -
Narrowing of the joint space (%)	Less than 25	25-50	50-75	> 75
Destruction of the arch laminae	+	+ +	+ + +	+ + -
Destruction of the joint surface (%)	-	Less than 25	25-50	> 50
Congruency disorders (dislocation, subluxation)	-	+	+ +	+ + +
Ankyloses / Lysis	-	-	+ -	+ +

age of the patients ranged from 18 to 73 years, with 32 (43.24%) females, and 41 (56.76%) males. Morphological and immune-histological biopsy samples were taken from the psoriatic eruption and intact skin regions to detect structure changes and microorganism colonies characteristics, according to the defined antigenic properties. The skin biopsy fragments were treated by 10% neutral formaline. Further, the biopsy material was treated with spirit to be introduced in paraffin blocks, from which histological sections 4-6mcm in width were prepared.

The obtained histological preparations were studied using the "Olympus BX 51" microscope, „Olympus C 5050 Z" digital camera and "Olympus DP-Soft" software.

To compare the results of appropriate morphological and immunohistochemical studies the authors studied biopsy samples taken from the anterior abdominal wall skin in almost healthy patients (5 patients).

RESULTS AND DISCUSSION

The performed morphological and immunohistochemical studies of skin biopsy samples taken from the skin psoriatic eruption regions revealed general inflammatory infiltration in epidermis and dermis layers as well as numerous germ colonies under the stratum corneum in 49 (66.21%) from 74 patients with psoriasis. Also, 19 (25.67%) patients had germ colonies under the stratum corneum in their biopsy samples taken from the intact skin regions. Altogether,

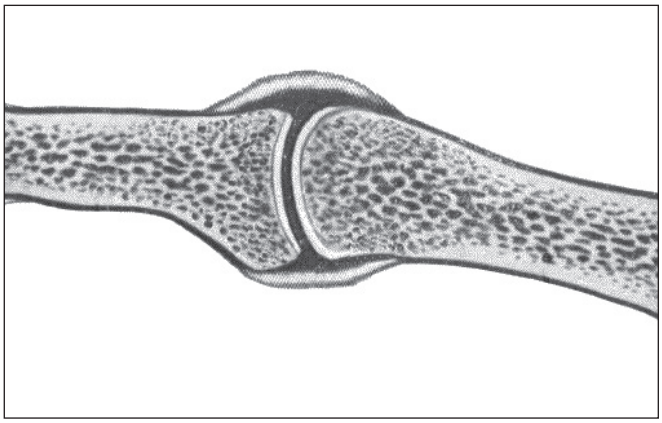

Fig. 1. Stage 1 (own figure).

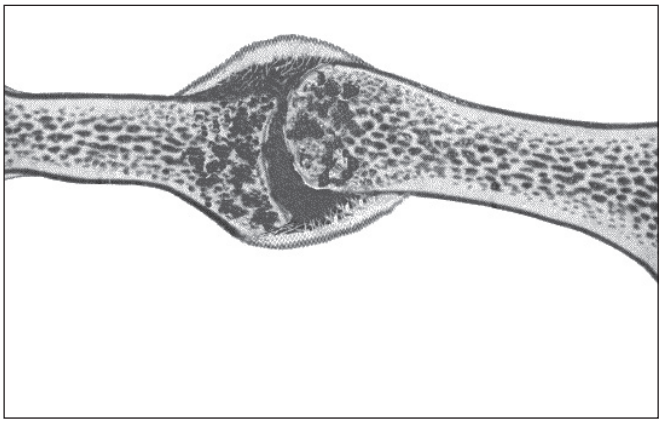

Fig. 2. Stage 2 (own figure).

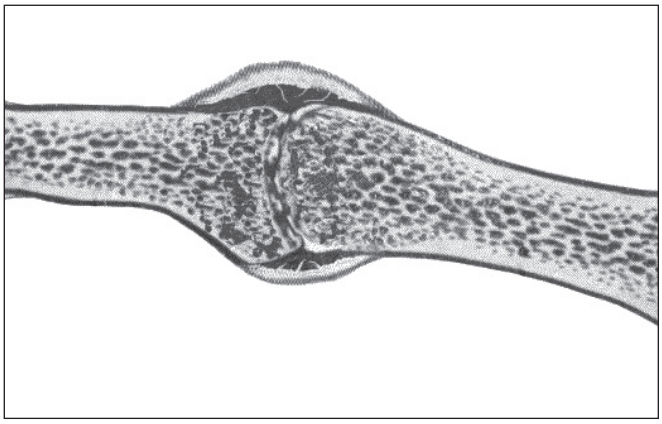

Fig. 3. Stage 3 (own figure).

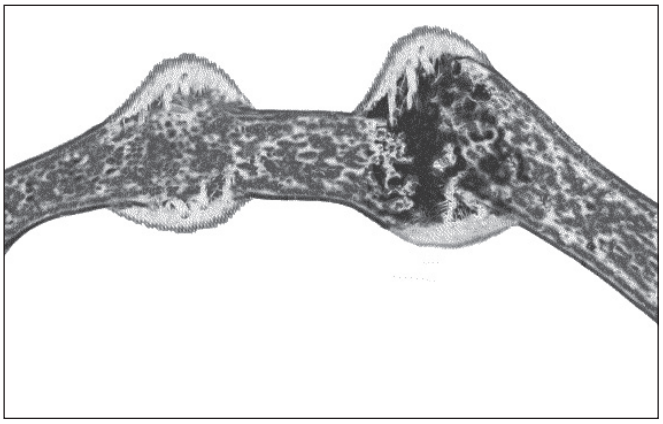

Fig. 4. Stage 4 (own figure).

the number of the detected microorganism colonies in the intact skin regions was considerably lower than that one in the skin psoriatic eruption regions. The general infiltration in the epidermis and dermis layers in the appropriate biopsy samples taken from the intact skin regions was absent.

The immunohistochemical trials were conducted, in order to establish the species composition of microorganism colonies which were detected under the stratum corneum in skin biopsy samples taken from the skin psoriatic eruption regions and intact skin of patients with psoriasis, and they showed that the appropriate microorganisms belong to Staphylococcus aureus species.

Examination of 25 (33.79%) from 74 examined patients with psoriasis didn't reveal present microorganism colonies under the stratum corneum in the biopsy samples taken from the skin psoriatic eruption and intact skin regions. The biopsy material taken from the anterior abdominal wall skin of the control group patients (5 almost healthy patients) didn't show microorganism colonies under the stratum corneum as well.

Totally the study includes 252 patients with PA, 147 (58.3 %) males and 105 (41.7%) females, which corresponds to 3:2 ratio. The grouping of patients according to the age, age period of the disease onset and disease duration are represented in table I.

The dysfunctions of muscular-skeletal system associated with PA most often appear within 1-3 years after the first manifestations of psoriasis (38.45%) and at the age period from 10 to 30 years (28.85%).

The most common muscular-skeletal dysfunction associated with PS is polyarthritis, which was diagnosed in 87.3% of all cases (220 patients), respectively mono- and oligo-arthritis in 12.7% of all cases (32 patients).

The data incidence of muscular-skeletal disorders according to localization are represented in table II.

Most often the muscular-skeletal disorders appear with psoriasis after the skin manifestations (79.76%), though they may precede them (4.76%) or develop simultaneously (15.48%).

The obtained roentgenological signs were grouped into the early and late ones. The early roentgenological manifestations of the PA are:

- its pronounced rate, affected structure, thickening of periarticular tissues;
- irregular narrowing of joint space;
- osteoporosis within the bone epimetaphyses;
- edge resorption of the hand and foot distal phalanges tuberosities (usuras).

The late roentgenological signs are:

- irregularity, thinning, disintegration of the joint surface end plates;
- linear periosteal layering in the hand and foot phalanges;
- focal or bone-like destruction and lysis of the epimetaphyses, which further lead to destructive dislocations and semi-dislocations;
- ankyloses

Table IV. Operative interventions according to the structural-functional dysfunctions

Structural-functional dysfunction stage	Operative intervention methods			
	Synovocapsulectomy. Tenoplasty	Arthroplasty	Endoprosthetics	Bone autoplasty
I	23	—	—	—
II	11	—	—	—
III	8	4	—	—
IV	—	3	31	2
Totally	42	7	31	2

Table V. Results of operative treatment according to the structural-functional dysfunction degree.

Structural-functional dysfunction stage	Treatment outcomes (%)		
	Good	Satisfactory	Unsatisfactory
Stage 1	95.7	4.3	—
Stage 2	92.2	7.8	—
Stage 3	76.3	18.5	5.2
Stage 4	68.1	20.1	11.8

Due to the complex and comparative analysis of the received results by the above-mentioned methods the authors have classified structural dysfunctions of muscular-skeletal system associated with psoriatic arthritis, the classification represented in table III.

The scheme of the offered classification is represented in figures: figure 1- stage 1; figure 2 – stage 2; figure 3 – stage 3: figure 4 – stage 4.

According to the above mentioned classification, the patients were divided into the clinical groups:

Group 1 – patients with initial lesions, the 1st stage of the muscular-skeletal structural disorders, the process activity: 1-2, joint insufficiency degree: 0 – 1. Such patients with absent deformities showed slight exudative-proliferative changes and initial muscular atrophy stage.

Group 2 – patients with initial and slight deformities, second stage of the muscular-skeletal system disorders, process activity: 1-2, joint insufficiency degree: 1-2.

Group 3 – patients with significant deformities, stage of structural muscular-skeletal system disorders, process activity: 1-2, joint insufficiency degree: 2-3.

Group 4 – severe deformities, ankyloses and lysis, significant function loss, stage 4 of the muscular-skeletal system structure disorders, process activity: 1-2, joint insufficiency degree -3.

According to the distribution of the patients into the above mentioned groups, appropriate operative interventions were conducted (table IV).

The results of operative treatment, conducted according to the degree of structural-functional disorders, are represented in table V. The analysis of the long-term outcomes of operative treatment showed their high efficiency. In patients with early stage synovectomies should be emphasized, conducted in the "classical" and arthroscopic ways. Synovectomies should be regarded as both treatment and prevention: excision of the pathologically changed synovial membrane decreases autosensibilization, improves trophics of the cartilage, which prevents severe destruction of the cartilage and epimetaphyses. Alongside, after such interventions we observe skin manifestations regress, prolonged remission and improvement of the patients' general condition, which is confirmed by the laboratory data stabilization. High efficiency of synovectomies was observed in group 1 and 2 of the patients with subacute and chronic inflammations.

Correcting osteotomies and endoprosthetics were conducted in group 4, and the analysis of long-term outcomes evidences about significant benefits of endoprosthetics.

CONCLUSIONS

1. Presence of numerous colonies of opportunistic microorganisms Staphyloccocus aureus under the stratum corneum in psoriatic skin eruption regions in most psoriasis patients with progressing dermatosis as well as the presence of these colonies under the stratum corneum of intact skin in some patients stipulates for the necessary skin debridement when planning operative treatment.
2. Complex pathogenetic treatment of patients with PA must include operative orthopedic interventions aimed at restoration of anatomical-biochemical dysfunctions of the muscular-skeletal system. Synovectomies and capsulosynovectomies represent the most effective treatment measures of treatment and prevention in patients of the 1st and 2nd clinical groups.
3. According to the conducted studies, the differential approach to operative treatment of patients with PA was elaborated, the approach considering the stage, form and spread of pathological process. Pre-operative preparation of the patients is of significant importance, as it must be pathogenetically determined and individualized.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

EFFECT OF ENDOSTATIN AND INSULIN-LIKE GROWTH FACTOR-1 ON ANGIOGENESIS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITH OBESITY UNDER THE INFLUENCE OF ZOFENOPRIL

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ABSTRACT

The aim: To study the dynamics of markers of angiogenesis based on insulin-like growth factor-1 (IGF-1) and endostatin, as well as to determine 6-month survival in patients taking zofenopril from the first day of AMI with and without obesity.

Materials and methods: using enzyme immunoassay, we determined the level of endostatin and IGF-1 in serum on days 1 and 12 in patients with AMI with the presence and absence of obesity, and a statistical processing of the data obtained.

Results: The relationship between obesity and angiogenesis indicators, both activators and inhibitors, was determined, and a significant relationship was found between zofenopril therapy and angiogenesis activator IGF-1. Differences in the survival of patients with complicated AMI were determined depending on the choice of ACE inhibitor in favor of a higher survival rate of patients who took zofenopril.

Conclusions: patients who underwent complicated AMI, taking zofenopril, have a higher survival rate during the 6-month follow-up period. Zofenopril stimulated angiogenesis in the examined patients, which was expressed in patients with and without obesity.

KEY WORDS: endostatin, IGF-1, acute myocardial infarction, obesity, zofenopril

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INTRODUCTION

Despite all the modern advances in medicine and technology, cardiovascular diseases currently occupy a leading place in the world in mortality [1].

Everyone knows such factors as: hyperlipidemia, dyslipidemia, diabetes, metabolic syndrome, smoking, sedentary lifestyle, hypertension, which play a very important role in the development and progression of atherosclerosis [2]. In recent years, more and more attention has been paid to the issues of endothelial dysfunction and angiogenesis of plaques as factors for the development and progression of coronary heart disease [3].

Today, metabolic syndrome is a serious public health problem. The main danger is associated with an increased risk of cardiovascular diseases, stroke, type 2 diabetes mellitus and a growing risk of mortality. Metabolic syndrome stimulates the occurrence of early atherosclerosis, its progress and accelerates the frequency of cardiovascular complications associated with atherosclerosis and diabetes mellitus [4]. More and more patients with obesity and overweight are registered worldwide. Obesity, as one of the indicators of metabolic syndrome, is a risk factor for the development of many diseases, such as: coronary heart disease, hypertension, diabetes mellitus [5].

In healthy people, the vascular endothelium plays a key role in maintaining homeostasis, since its functions include inhibition of blood coagulation in the vessel lumen, vasodilation of the vascular wall, regulation of the coagulation processes, thrombosis and fibrinolysis, immune and inflammatory reactions, neovasculogenesis and prevention of proliferation of smooth muscles, [6]. However, endothelial function may be impaired, due to which an imbalance of angiogenesis factors occurs, leading to a state in which vasoconstriction, thrombosis, proliferation of vascular smooth muscle cells and the formation of atherosclerotic plaques occur [7].

When establishing endothelial dysfunction, an imbalance between pro- and anti-angiogenic factors contributes to the occurrence of hemorrhagic, immature capillaries inside the vessel wall or inside nascent plaques. Thus, the regulation of angiogenesis appears to be a key factor in the spread of coronary artery disease and atherosclerotic plaque rupture [3].

In this study, angiogenesis factors such as insulin-like growth factor-1 (IGF-1) and endostatin were considered. IGF-1 is a product of the IGF-1 gene, which is located on chromosome 12 [8]. On the first and second exons, there are two sites for running transcripts showing the results in two different carboxyl terminal domains for IGF-1 (Ea and Eb). Eb transcripts are mainly expressed in the liver. In addition, IGF-1 is also synthesized in the kidneys, car-

diomyocytes, smooth muscle vascular cells, fibroblasts, etc. IGF-1 was identified as a valuable indicator of impaired glucose tolerance, and therefore its association with the presence of coronary atherosclerosis was suggested. Several studies have evaluated the relationship between the level of IGF-1 and the severity of atherosclerosis [9]. It has now been established that endothelial dysfunction is an early event in atherogenesis that precedes intimal thickening and the formation of atherosclerotic plaques. A feature of endothelial dysfunction is the reduced bioavailability of the anti-atherosclerotic nitric oxide molecule (NO). In the course of research, there is evidence that IGF-1 is a powerful vasodilator [10]. In endothelial cells, IGF-1, interacting with its receptor, stimulates the formation of NO, contributing to the regulation of vascular tone [11].

Endostatin is a fragment of the C-terminal region of collagen XVIII [12]. Endostatin is found in the walls of blood vessels (elastic fibers) and membranes with a strong effect of modulating angiogenesis and is a component of almost all epithelial and endothelial membranes in the human body [13]. According to known data, endostatin induces apoptosis of endothelial cells [14], plays an important role in the adhesion of endothelial cells [15] and impairs the maturation of blood vessels during wound healing, which leads to a decrease in angiogenesis [16].

Obesity, low physical activity, diabetes mellitus can affect the level of endostatin in serum [17, 18]. Disrupted endostatin levels in the blood serum are associated with cerebrovascular diseases, organ damage in hypertension [19]. Due to the serum level of endostatin, mortality from cardiovascular diseases in people with ischemic heart disease can be predicted [20].

According to international recommendations, angiotensin-converting enzyme (ACE) inhibitors are considered to be one of the most important drugs for treating patients with a high risk of developing cardiovascular diseases, as well as necessary drugs for the prevention of complications after acute myocardial infarction (AMI). Zofenopril is a highly lipophilic ACE inhibitor that is characterized by sustained inhibition of the progression of heart failure. As was shown in preclinical studies, zofenopril has cardioprotective properties and affects left ventricular remodeling in myocardial damage [21]. It is known that therapy with zofenopril may be an important support for ensuring a favorable prognosis for patients with a high risk of cardiovascular diseases after AMI [22].

THE AIM

Objective: to study the dynamics of angiogenesis inhibitors and activators based on IGF-1 and endostatin, as well as 6-month survival in patients with and without obesity after AMI under the influence of treatment with zofenopril.

MATERIALS AND METHODS

The study involved 105 patients with AMI and concomitant obesity who were treated in the infarction department of Kharkiv City Clinical Hospital No. 27 (mean age 64.6 ± 7.4 years). 55 patients had AMI and concomitant obesity, and 50 patients with AMI without obesity. The studied groups were comparable by sex and age. The control group consisted of 20 healthy individuals of appropriate age and gender.

AMI was diagnosed in accordance with the recommendations (recommendations of the ESC for the treatment of acute myocardial infarction in patients with ST-segment elevation 2017).

The anthropometric indices were determined: height (cm), body weight (kg), body mass index (BMI), which was calculated by the formula: BMI = body weight, kg / height, m^2. Obesity was diagnosed as recommended (American Association of Clinical Endocrinologists and American College of Endocrinological Clinical Practice for Comprehensive Medical Care for Obese Patients, 2016). With a BMI of 18.5 to 24.9 kg/m^2, normal weight, from 25.0 to 29.9 kg/m^2, increased body weight, and from 30 kg/m^2 and more, obesity.

There were 44 patients with complicated AMI: post-infarction angina (n = 15), atrial fibrillation (n = 12), ventricular fibrillation (n = 2), atrioventricular blockade (n = 4), pulmonary edema (n = 4), cardiac asthma (n = 2), thromboembolism of pulmonary artery (n = 3), repeated myocardial infarction (n = 2).

To determine the levels of IGF-1 (MEDIAGNOST, Germany), endostatin (BIOMEDICA, Austria), an enzyme immunoassay was used. Indicators of IGF-1 and endostatin were determined on the first and twelfth days of AMI.

Standard therapy was used for treatment of patients with AMI: percutanieus intervention, thrombolytic therapy, anticoagulants, antiplatelets, β-blockers, and also ACE inhibitors (zofenopril or enalapril). Zofenopril was used according to the scheme: 1-2 days of AMI, 7.5 mg 2 times a day, 3-4 days 15 mg 2 times a day, after 5 days 30 mg 2 times a day. Enalapril was used according to the scheme 10 mg 2 times a day.

Statistical processing was carried out using the program STATISTICA 10.0. Data are given as M ± m, where M is the mean and m is the standard error of the mean. The risk ratio (HR) and 95% confidence intervals (CI) were calculated using the Cox proportional risk regression model. Considering the different periods of observation for the patients examined, the relative risk of mortality from cardiovascular diseases was assessed using a time-dependent regression model and corresponding survival curves. Statistically significant differences were considered at $p < 0.05$.

RESULTS AND DISCUSSION

As a result of a 6-month follow-up in a group of patients with complicated AMI who received the recommended therapy with zofenopril, compared with another, similar in all parameters, group of patients who received therapy with enalapril, the HR risk ratio was 0.81 (0.61-0.95, P = 0.041). As can be seen, comparing the data of Figure 1 and Figure 2, the 6-month survival in patients with complicated AMI who received zofenopril was significantly higher than

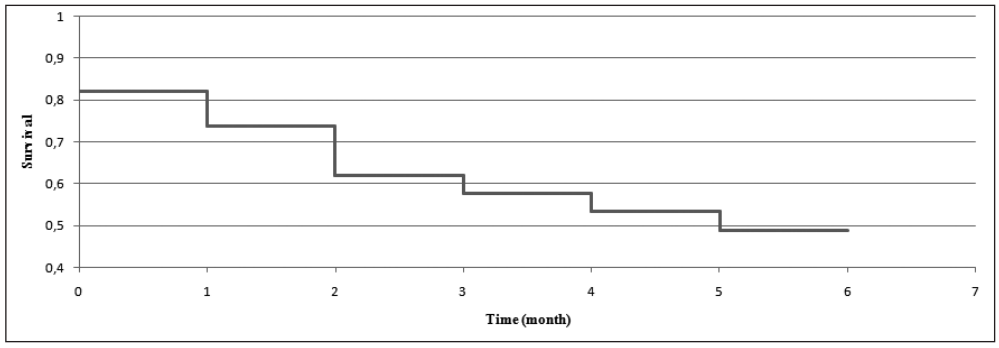

Fig. 1. Survival in patients with complicated AMI without obesity who received therapy with enalapril

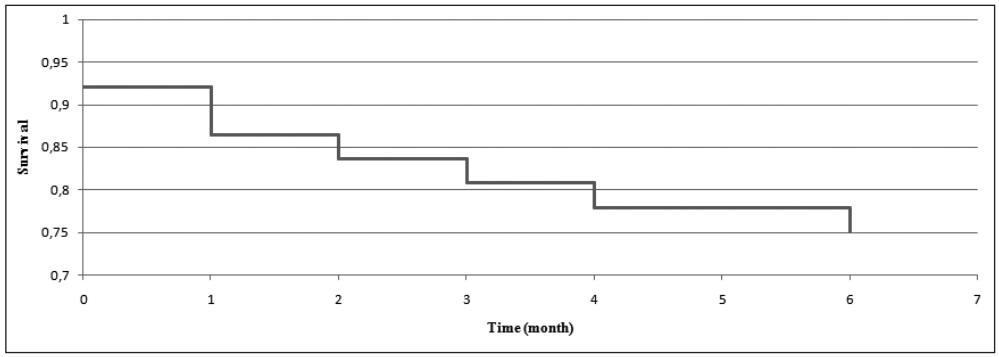

Fig. 2. Survival in patients with complicated AMI without obesity who received therapy with zofenopril.

Table I. Dynamics of indicators of angiogenesis in patients with complicated acute myocardial infarction in patients without obesity

Drug	Endostatin in 1 day, pmol/l	Endostatin in 12 day, pmol/l	IGF-1 in 1 day, ng/ml	IGF-1 in 12 day, ng/ml
Zofenopril	152,49±5,75	131,19±5,31*	135,07±8,04	159,07±8,57*
Enalapril	157,11±6,53	160,91±5,84	139,19±7,88	119,66±5,92*

*- p<0,05, as compared with 1 day of AMI.

Table II. Dynamics of indicators of angiogenesis in patients with complicated acute myocardial infarction in patients with obesity

Drug	Endostatin in 1 day, pmol/l	Endostatin in 12 day, pmol/l	IGF-1 in 1 day, ng/ml	IGF-1 in 12 day, ng/ml
Zofenopril	142,78±5,12	138,37±5,66	174,07±11,15	202,07±12,22*
Enalapril	149,11±5,84	144,11±5,68	171,81±10,48	182,66±7,32

*- p<0,05, as compared with 1 day of AMI.

in the group of patients who received enalapril. These figures show that the greatest number of deaths when using zofenopril occurred in the first month of AMI (30.77% of the total), and in the treatment with enalapril – in the second month after the coronary event (28.12% of the total) . In the future, mortality rates decreased to a minimum in the last 6th month of observation.

A dynamic change in the indices of the activator and angiogenesis inhibitor in patients with complicated AMI under the influence of zofenopril and enalapril therapy depending on the presence of obesity was also determined.

From Table I it is seen that in patients with complicated AMI without obesity who received therapy with zofenopril, there was a statistically significant decrease in the level of endostatin (152.49 ± 5.75 pmol / l and 136.19 ± 5.31 pmol / l, respectively, p <0.05), as well as an increase in the level of IGF-1 (135.07 ± 8.04 ng / ml and 169.07 ± 8.57 ng / ml, respectively, p <0.05) . The dynamics of endostatin in patients who received enalapril therapy was not statistically significant, however, there was a decrease in the level of IGF-1 (139.19 ± 7.88 ng / ml and 119.66 ± 3.92 ng / ml, respectively, p <0.05).

In Table II, the data of patients with complicated AMI and concomitant obesity are presented, there is a statistically significant increase in the level of IGF-1 in patients who received zofenopril (174.07 ± 11.15 ng / ml and 202.07 ± 12.22 ng / ml, respectively, p <0.05). There is also a significant difference between the indices of IGF-1 level in patients with obesity and without obesity at all stages of observation (135.07 ± 8.04 ng / ml and 174.07 ± 11.15 on the first day of acute MI, as well as 159, 07 ± 8,57 ng / ml and 202,07 ± 12,22 ng / ml for 12 days of acute MI, respectively, p <0.05), compared to patients receiving therapy with zofenopril. The difference of the other indicators was not statistically significant.

In Ukraine, as well as throughout the world, cardiovascular diseases are the most common pathology and the leading cause of death among patients [1]. The combination of pathology of the cardiovascular system and obesity further aggravates the patient's condition and increases the risk of complications and mortality in such patients [23].

Studies of markers of angiogenesis in patients with AMI and the data obtained give us a reason to talk about their effect on the progression of cardiovascular diseases. The diagnostic role of endostatin and IGF-1 in AMI is beyond doubt [24, 25].

Scientists have conducted studies where it was reported that the level of IGF-1 may increase with obesity [26, 27]. Other studies [28] show that the level of IGF-1 does not increase or may even be decreased [29]. In our study, it is clearly seen that the level of IGF-1 in patients with AMI and obesity was increased in comparison with a comparable group of patients without obesity.

Many scientists have noted the beneficial effect of ACE inhibitors for the further survival of patients with AMI [30, 31]. We studied the member of this group of drugs – zofenopril. The beneficial effect of zofenopril on angiogenesis was revealed: an increase in the level of IGF-1 in all categories of patients, as well as a decrease in endostatin in patients without obesity. Occurrence a higher survival rate was also registered in patients who took zofenopril since the AMI, which is consistent with the results of other studies [22, 32].

CONCLUSIONS

1. The level of IGF-1 in patients with AMI and obesity is significantly higher than the level of IGF-1 in patients with AMI without obesity.
2. Treatment with zofenopril compared to the use of enalapril has a positive effect on the activation of angiogenesis in the form of an increase in the level of IGF-1 in all categories of patients.
3. In patients who were under the treatment of zofenopril from the first day of AMI, there are higher rates of survival in the 6-month follow-up period.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

EFFECT OF PRIMARY STENTING OF CORONARY ARTERIES ON CLINICAL COURSE AND REMODELING OF THE LEFT VENTRICLE IN PATIENTS WITH ACUTE ST SEGMENT ELEVATION MYOCARDIAL INFARCTION (RESULTS AFTER 12 MONTHS)

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ABSTRACT

The aim: To determine the predictive effect of primary stenting of coronary artery on the clinical course and features of left ventricular remodeling in patients with acute myocardial infarction (MI) with ST segment elevation and LV diastolic dysfunction (DD) in the remote period (12 months).

Materials and methods: The study included 80 patients with STEMI who undergo primary PCI (group 1) and 20 patients with STEMI who did not undergo myocardial revascularization (group 2). In both groups, the levels of NT-proBNP were determined, echocardiography, bicycle ergometry; quality of life was determined according to SAQ questionnaire scales; marked cardiovascular complications. Patients were re-examined after 12 months.

Results: The average level of NT-proBNP for 5 days and 12 months of patients in group 1 significantly decreased – 434.6 ± 36.3 and 122.8 ± 4.13 g / ml ($p < 0.001$), indicating a less pronounced late remodeling of LV in patients undergoing revascularization with STEMI. Diastolic function was evaluated by E/A and DT. After 12 months, the 1st group was markedly lower than E/A and DT compared to the 2nd – 0.76 ± 0.03 and 198 ± 4.7 m / s and 1.49 ± 0.01 and 135 , 3 ± 2.91 m/s, respectively ($p < 0.05$), which characterizes a decrease in the parameters of the DD by the type of the relaxation processes of the left ventriculi. Patients in Group 1, according to SAQ scales, had better quality of life and higher exercise tolerance at I-II level than patients without revascularization.

Conclusions: In patients with STEMI, after a primary stenting of coronary artery, a significantly lower NT-proBNP level and less pronounced DD manifestations in the long-term (12 months) period demonstrated a better tolerance to physical activity and improved quality of life, as determined by the SAQ questionnaire and a lower rate of development cardiovascular complications.

KEY WORDS: ST segment elevation myocardial infarction, diastolic dysfunction, brain natriuretic peptide, primary stenting of coronary artery

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INTRODUCTION

Initial coronary stent implantation improves the prognosis for patients with acute myocardial infarction (MI), influencing the left ventricle (LV) remodelling via improvement of diastolic function, which prevents the heart failure (HF) with reduced ejection fraction in the future [1]. Possibilities to diagnose left-ventricular diastolic dysfunction (DD) are rather limited, thus, this condition often remain not diagnosed during significantly long time. There are no specific clinical criteria featuring this pathology. However, taking into consideration the pathophysiological mechanisms related to LV diastolic pressure increase, which leads to the increasing of the Brain Natriuretic Peptide level, it was suggested that the level of the N-final fragment – predecessor of the Brain Natriuretic Peptide (NT-proBNP) reflects the severity of DD in patients with LV preserved ejection fraction [2]. Analyzing the fluctuation of the NT-proBNP level, one can evaluate the effectiveness of the conservative and invasive treatments for these patients. In accordance with the references, in patients with the ST-elevation myocardial infarction (STEMI) the NT-proBNP concentration is lower after performance of initial percutaneous coronary intervention (PCI) than for patients who received only conservative therapy [3]. Analysis of the NT-proBNP level in the group with hemodynamically significant stenosis showed that it definitely increased in case the quantity of the damaged vessels increased, reflecting the size of the myocardial ischemia. Correspondingly, patients having one damaged vessel definitely had the lower NT-proBNP concentration, than those with two- and three-vessels damage [4]. Thus, the myocardial revascularization has a significant meaning for the prognosis improvement. Therefore, definition of the NT-proBNP level is an extremely important value while detecting the symptomless dysfunction of LV, as well as while estimating the prognosis and monitoring of heart failure for these patients [5,6].

In accordance with the theory of 'open artery', the patency of infarction- dependent coronary artery plays a key role in the LV re-modelling in patients with STEMI [7,8]. Renovation of the blood flow reduces the level of

myocardial necrosis and spreading of infarction zone, thus improving prognosis. Besides, the particular importance is given to the modern 'microvasculature opening' theory. Research results showed that 30% of patients with STEMI, who received PCI, notwithstanding the full renovation of patency in infarction-dependent artery (IDA), got the dilatation of LV only 6 months after MI [9,10]. The reason for this may be the absence of the blood flow in coronary microcirculation, so-called 'no-reflow' phenomenon, which, notwithstanding the fully renovated IDA patency, leads to bigger tissue necrosis and higher frequency of complications [11-16]. Thus, it is important to identify and influence in time on main factors, predicting the negative remodeling of LV after MI, namely: absence of PCI for blood flow renovation through IDA and microvasculature; MI of the front LV wall; elevated NT-proBNP level and symptoms of heart failure at the moment and when a patient is discharged from the hospital [3].

The evaluation of echo-cardiographic DD parameters is really important for patients with STEMI on the early stage after PCI, as it can influence negatively on the prognosis. Among others, this is the detection of the restrictive type DD with the apparent decrease in the deceleration time of the early diastolic transmitral flow (DT) to less than 130 ms [17-19]. Several researchers showed that notwithstanding the correlation of the speed of early to the advanced transmitral flow (E/A), the DT slowing down in the post-infarction patients is the sign of the elevated pressure in the LV and leads to the increase of MI size [20-22]. This data is confirmed by Nijland F. and al., who informed that survival rates within one and three years after MI were only 50% and 22% correspondingly [23]. Poulsen S. and al. informed about the cardiologic mortality during the year for 43% of the patients with the pseudo-normal values of the restrictive DD type at the beginning of the MI onset [24].

Thus, it is reasonable to suggest that mechanic reperfusion with preservation of the IDA patency may suspend the process of the pathological LV remodeling or at least mitigate its negative influence on the LV remodeling, and improve the prognosis for those patients.

THE AIM

Aim of work – to evaluate the prognostic influence of the initial PCI on the 12 months left-ventricle remodeling in patients with STEMI and diastolic dysfunction.

MATERIALS AND METHODS

The research included 100 patients with confirmed STEMI, who were divided into two groups: 80 patients (1st group) who have been performed PCI. The proportion of males was 68.1 %, females – 31.9 %, the mean age – (56.7 ± 7.3) years. The 2nd group consists of 20 patients, who did not receive myocardial revascularization. This group included 75 % males, 25 % females, the mean age was (66.1 ± 7.3) years. Revascularization for the 2nd group was not performed due to technical difficulties of CA stenting, late hospital admission (outwith the reperfusion window time), patients refusals and so on. Depending on the PCI performing time, patients of the 1st group were split into three subgroups. PCI was performed within 12 hours of the onset of MI symptoms in patients of 1A subgroup (n=56), from 12 to 24 hours in patients of 1B subgroup (n=16) and after 24 hours in patients of 1C subgroup (n=8).

All patients were examined on the 5th day from the beginning of the MI and after 12 months (apart from coronary angiography). The traditional cardiovascular risk factors were identified as well as STEMI complications (MI relapse, rhythm and conductivity disorders, acute heart failure and sudden death). Apart from it, on the 21st-28st day the bicycle exercise was performed to establish the functional class of post-MI angina by the Canadian Cardiovascular Society angina (CCSA) classification.

Coronary angiography data (infarction-dependent artery localization, quantity of the damaged vessels) and PCI results were analyzed. Effectiveness of revascularization was evaluated by the TIMI coronary grade flow.

NT-proBNP plasma level was measured.

Transthoracic echocardiography was performed in accordance with the recent 2016 American Society of Echocardiography (ASE)/European Association of Cardiovascular Imaging (EACVI) recommendations in the prevalence and grade of DD. Diastolic dysfunction was diagnosed by transmitral early and advanced diastolic filling ratio (E/A) and deceleration time (DT) of the early transmitral flow.

Quality of life was estimated according to the Seattle Angina Questionnaire (SAQ) by the following scales: PL (physical limitation), AS (angina stability), AF (angina frequency), TS (treatment satisfaction) and DP (disease perception) [25].

The treatment was performed in accordance with the ESC guidelines (2017) for Acute Myocardial Infarction in patients presenting with ST-segment elevation.

All patients received the ASA loading dose – 300 ng; Clopidogrel – 300 mg, and in case of PCI – additional loading dose of Clopidogrel – 300 mg in 30 % of patients and Ticagrelor – 180 mg in 70 % of cases with the following intake of the double anti-aggregate therapy in the standard dosage during 12 months; statin-therapy (Atorvastatin 40-80 mg or Rosuvastatin 20-40 mg), and the accompanying background medicinal therapy in the form of: 90.2 % – angiotensin-converting enzyme inhibitors (ACEi); 9.8 % – Angiotensin II Receptor Blocker (ARB); 19.6 % – mineralocorticoid receptor antagonist (MRA); 82.4 % – β- adrenergic blocking agents; 4.8 % – amiodarone; 45.3 % – nitrates/sidnonimines. Besides, all patients of the 2-nd group received the low-molecular anticoagulants during the first 8 days, and the oral anticoagulants – 4.7 % of the patients from the both groups, and the patients with the diabetes received the oral glucose-lowering medications – 32.0 %, insulin – in 5.9 %.

Statistical processing of the data was performed with the help of the Statistica software and Microsoft Office Excel 2013 packet. The data is represented in the form of the

average meaning and the standard error of the mean (M ± m). The authenticity of the differences between the groups according to the quantitative characteristics was evaluated with the help of the Student's t-criterion (in case of normal distribution). Differences were supposed to be authentic in case the significance level was 95 % (p<0.05).

RESULTS AND DISCUSSION

Risk factors in the 1st group were: arterial hypertension – 70.5 %, type 2 diabetes mellitus – 41.5 %, smoking habits – 40.9 %, hypercholesterolemia – 56.8 % and overweight (mean body mass index was 29.4 kg/m^2). The 2nd group revealed the similar structure of the risk factors: arterial hypertension – 80.5 %, type 2 diabetes mellitus – 48.5 %, smoking habits – 49.9 %, hypercholesterolemia – 66.8 % with average body mass index of 29,1 kg/m^2. Medical history of the 1st group reveled previous MI in 9.1 %, coronary artery bypass grafting or PCI – in 6.8 %, angina of the II–III CCSA class – in 15.9 %, atrial fibrillation – in 4.7 %, stroke – in 4.6 % participants; in the 2nd group: angina of the II–III CCSA class – in 21.4 %, previous MI – in 7.8 %, atrial fibrillation – y 8.1 %. Thus, in accordance with cardiovascular risk factors and the anamnestic data the two groups under research were alike.

Following the coronary angiography, among patients of the 1st group the infarction-dependent artery were localized in proximal and medial segments of left anterior descending artery (LAD) in 21 and 18 patients (26.25% and 22.5 %) correspondingly, medial segment of circumflex artery (CfA) – 4 (5 %) patients, right coronary artery (RCA) – 37 (46.25 %) patients. In the 2nd group LAD was occluded in 9 (45 %) patients, CfA – in 1 (5 %) patient, RCA – in 10 (50 %) patients.

In the 1st group multi-vascular lesioning of CA was observed in 16 (20 %) patients, two-vessels lesioning – in 24 (30 %), lesioning the one infarction-dependent artery was in 40 (50 %) cases. Complete revascularization (all arteries with stenosis > 70 % were revascularized) was performed as the initial PCI in 45 patients (56.25 %). Incomplete revascularization – stenting of the infarction-dependent artery only – was performed in 35 patients (43.75 %) with the further complete revascularization after the 21st day from the onset of MI by the PCI in 20 (57.1 %) and coronary artery bypass grafting – in 2 (5.7 %) patients. In 91 % of cases we achieved TIMI 3 flow, in 9 % of patients – TIMI 2 flow.

In the 2nd group the multi-vascular CA lesioning was observed in 50% of patients, two-vessel lesioning – in 30% and lesioning of only one infarction-dependent artery – in 20 % of patients.

NT-proBNP level on the 5-th day from the hospital admission corresponds to the 2-nd spike in patients with STEMI and authentically reflects the LV re-modeling process. Average NT-proBNP level in patients of the 2nd group on the 5-th day was (1182.6 ± 280.8) pg/ml, and after 12 months lowered to (629.3 ± 82.2) pg/ml (p > 0.05). Patients of the 1st group had the significantly lower NT-proBNP level on the 5th day and after 12 months correspondingly – (434.6 **± 36**.3) i (122.8**± 4**.13) pg/ml (p < 0,001), which shows the positive influence of myocardial revascularization on the LV remodeling process after the MI development.

The E/A ratio and DT in patients of the 1-st group were (0.87 ± 0.05) and (186 ± 2.8) ms correspondingly, and after 12 months – (0.76 ± 0.03) and (198 ± 4.7) ms correspondingly, which certifies about the improvement of LV relaxation processes (p <0.05). The E/A ratio and DD values of patients of the 2-nd group appeared to be as follow – (1.37 ± 0.03) and (145.75 ± 2.91) ms at the admision, and (1.49 ± 0.01) and (135.3 ± 2.91) after 12 months correspondingly, which is characteristic to the DD increase according to restrictive type (p<0.05). After 12 months the significant difference between two groups was observed (p<0.01).

Results of the evaluation of diastolic function and NT-proBNP level in patients of three sub-groups (1A-1C) showed that postponed revascularization (more than 24 hours from the MI onset) has led to more significant LV remodeling in the form of the restrictive type of DD and higher levels of NT-proBNP in the 12 months period after the MI.

Comparative analysis of CA lesioning and NT-proBNP level in the 1st group showed the following results: in case of the multi-vascular CA lesioning the NT-proBNP was (518.4 ± 19.9) pg/ml; two-vessels – (470.0 ± 10.5) pg/ml; one-vessel lesioning – (369.8 ± 10.69) pg/ml (p < 0.05 between groups). Thus, presence of the residual ischemia in case of the three-vessels or two-vessels CA lesioning, even after the successful PCI, is accompanied with the higher NT-proBNP level, which in future influences the speed and processes of the LV remodeling. That is why it is important to evaluate the full revascularization after 12 months by the estimation of NT-proBNP level. The group of patients discharged from the hospital with incomplete revascularization (coronary artery stenosis was present in more than 50% of arteries with the diameter > 2 mm) comparing to the patients with full revascularization had the tendency to lowering of NT-proBNP level (126.5± 7.1) pg/ml and (121.1± 5.2) pg/ml correspondingly, which can certify about the effectiveness of full myocardium revascularization in patients with STEMI at hospital discharge.

Seattle Angina Questionnaire (SAQ) data showed that 1st group patients had better quality of life values comparing to the 2nd group in distant period after MI.

Patients underwent the bicycle exercise tolerance test on the 21st-28th day after the STEMI beginning in order to define the angina functional class (FC). All 2-nd group patients had III–IV FC angina, while the 1st group patients had I–II FC. Thus, patients with undergoing PCI had better exercise tolerance to the physical loading. Unlike the patients of the 2nd group whose exercise tolerance did not change within 12 months.

During the time in hospital the cardiovascular complications were evaluated. Group without PCI: 2 (10 %) patients died, in 1(5 %) complete AV heart block developed and in

1(5 %) was detected ventricular tachycardia. PCI group: 1(1.25 %) patient with inferior MI after PCI developed transient AV heart block, and in one case paroxysm of supraventricular tachycardia was detected, which was treated by intravascular injection of Amiodarone (1.25 %).

Within 12 month the percentage of current complications for the group with the performed PCI was 15% (3 (3.75%) patients with atrial fibrillation, 5 (4%) – hospital admission due to worsening of HF, 1(1.25%) – subsequent MI, 3 (3.75%) – unstable angina. In group without PCI the quantity of cardiovascular complications was 66.7%. One (5.5%) patient died (recurrent MI), 6 (33.3 %) patients had hospital readmission due to heart failure (50% of them have atrial fibrillation), 5 (27.8%) patients have unstable angina ($p < 0.05\%$).

CONCLUSIONS

1. Initial PCI in patients with STEMI leads to the significantly lower NT-proBNP level in the 12 months period, which reflects processes of pathologic LV remodeling, comparing to the group without revascularization.
2. Echo-cardiolographic features of diastolic function within 12 months in patients with STEMI and PCI was characterized with improvement of LV relaxation impairment and reduction in numbers of akinetic and hypokinetic segments. The group without PCI had markedly distinctive LV remodeling in the form of restrictive type of DD and reduction of LV ejection fraction from 55.0±4.5 to 45.3± 0.6%.
3. During the first year of observation the patients with STEMI and PCI showed higher quality of life, with the sufficient exercise tolerance of the 1-2 angina FC, comparing to the group without revascularization (3-4 angina FC), and lower quality of life, according to SAQ scales.
4. In the group of patients without PCI there were more cardiovascular events (66.7%) comparing to PCI group (15%) ($p<0.05\%$) within 12 months from the STEMI onset, which certifies about the worse prognosis in patients with STEMI without revascularization.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

STATE OF DENTAL HEALTH OF CHILDREN IN UZHHOROD AND THE WAY OF THEIR NUTRITION

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ABSTRACT

The aim: To study and evaluate the condition of the dental health of children of school age and the characteristics of their food.

Materials and methods: Statistics on the dental health of children in Transcarpathian region for the years 2009-2018 were analyzed with the application of statistical, sociological and clinical methods and the data on dental health and way of nutrition of 163 children in Uzhgorod were determined and analyzed.

Results and conclusions: Reduction of 28.9% of coverage of children with preventive dental examinations was determined with the need for mouth cavity sanation of 45.93%. Redevelopment was held for 91.7% of those who needed help.

From 10% to 50% of the examined children in different age groups have caries. Up to 10% of children between 11 and 15 years old have teeth removed. It was determined that nutrition of children with caries diagnosed is not rational.

KEY WORDS: children, caries, nutrition, stomatological care

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INTRODUCTION

In modern ecological and socio – economic conditions, there is an increase in the incidence of children whose organism is sensitive to harmful factors. One of the integrated indicators of the health of the pediatric population is dental morbidity. The condition of the oral cavity of the baby has a major impact on overall health. At this stage, the dental health of Ukraine's child population is worsening every year and is characterized by a high prevalence of caries. Studies have shown a significant prevalence of dental caries in children at different ages [1, 2, 3, 4].

On average, children in school age have 3-4 permanent teeth, and at the time of graduation, one in five children has one permanent tooth removed. The incidence of periodontal disease is also increasing rapidly [5]. This is due to the peculiarities of the structure of nutrition, the level of hygienic skills, the effectiveness of remedial measures, including fluoroprophylaxis [6].

Monitoring of dental health of children and adolescents of different regions of Transcarpathian region has shown that the condition of the oral cavity in children of all age groups is satisfactory, but there is a risk of periodontal diseases [7,8,9,10]. Identical studies were conducted in Uzhgorod [11].

One of the important factors affecting the dental health of children is the nature of their diet. According to publications in the nature of nutrition of the infant population of Ukraine, over the last two decades, there have been negative changes, and the deterioration of the structure of productive diets of children, their imbalance by major nutrients [12].

The study of the prevalence of dental diseases in the pediatric population and their relationship with nutrition is at the heart of rational nutrition and dental care planning. Correct nutrition plays an important role in preventing caries. This issue should be considered in two aspects: 1) the indirect influence of nutrients through the formation of organs and systems (including the tooth-jaw apparatus) and 2) the direct effect of food on the tissues of the tooth and periodontal. The state of the solid tissues of the tooth, the pH of saliva and plaque, the rate of tartar deposition, and the stimulation of salivation, depend on the nature of the food. The tooth is a semipermeable membrane, the permeability of which depends on the physicochemical features of the surrounding media. That is, a certain orientation of the metabolic processes, which contributes to carious tooth damage [13].

An important component of a person's diet are macro-, micro-, and ultramicroelements that provide remineralization of tooth enamel. Therefore, one of the directions of prevention of demineralization of enamel (reverse process) and related complications is to balance the content of chemical elements in food. Particularly important among macronutrients are compounds of calcium and phosphorus. Among the trace elements of food, ions of aluminum, copper, zinc, iron, manganese and tin promote the incorporation and retention of calcium and fluorine in enamel and inhibit the production of acids by plaque [14,15].

Studies have been conducted that indicate a direct correlation between the consumption of easily digestible carbohydrates and the intensity of caries development [16].

Carbohydrate intake is an important etiological factor in the development of caries. . An abundance of fast food, poor quality, high sugar and simple carbohydrate-based diets in lower income neighborhoods predispose children to the development of plaque, dental decay and caries [17,18]. In the prevention of caries a special place belongs to vitamins, which are found mainly in fresh vegetables and fruits [19].

THE AIM

To study and evaluate the dental health status of school children and the nutritional status of these children.

MATERIALS AND METHODS

The study was based on WHO recommendations for dental examination [20], using modern methodological approaches to conducting preventive dental examinations [21, 22].

According to WHO recommendations, the following levels of caries intensity are distinguished: 0-1.1% – very low, 1.2-2.6% low, 2.7-4.4% average, 4.5-6.5% high, 6.6% or more is very high. And also the following degrees of caries prevalence: 0-30% – low, 31-80% – average, 81-100% – high prevalence.

The study was conducted in Uzhhorod in March – June 2019.

The following methods were used in the study:

-statistic: for the purpose of analyzing statistics of preventive examinations of the child population of Transcarpathian region, including in Uzhgorod, for 2009 – 2018. Data of sectoral statistical reports for the specified period were used as the materials of the research;

-sociological: in order to conduct a survey, according to a specially designed questionnaire, to study the way of feeding children.In total, 400 questionnaires of sociological study were handled;

-clinical: preventive dental examination of school-age children. Observation data were recorded in the oral examination charts in children. The prevalence, intensity, and the degree of caries compensation, the increase / reduction of the caries intensity, were used to evaluate dental caries. The surveyed children were divided by gender and age group.

The total number of surveyed and conducted preventive dental examination of 163 children of middle and high school age in Uzhgorod: 9 years – 30 (18,4%), 10 years – 33 (20,2%), 11 years 32 (19,6%), 12 years – 38 (23.4%), 15 years – 30 (18.4%).Of the total number of examined children 48.3% were boys and 51.7% – girls. General population amounted to 10526 children of appropriate age residing in Uzhhorod.

Statistical analysis of the obtained data was carried out using special packages of application statistics Microsoft office EXCEL.

RESULTS AND DISCUSSION

At the beginning of the study, the statistical results of dental preventive examinations of the pediatric population of the Transcarpathian region, including Uzhgorod, for the period 2009 – 2018 were analyzed.

It is established that every year the number of children covered by preventive examinations in the region decreases. Thus, in 2018 the number of children covered by preventive dental examinations was 7.1% lower than in 2009. The same tendency is found in Uzhgorod, where in 2009 there were 19282 children undergoing preventive dental examinations and 13706 children in 2018: 5576 fewer children. At the same time, the share of the pediatric population covered by preventive dental examinations decreased from 79.97% to 60.22%: by 19.75% less.

It should be noted that during the specified period in the region the share of children requiring rehabilitation increased by 25.84%. In Uzhgorod, per 1,000 children surveyed, the proportion of those requiring rehabilitation decreased from 730.03 in 2009 to 459.35 in 2018, representing 62.92% of the 2009 figure.

Remediation of the oral cavity in 2009 and 2018, respectively, was carried out in 90.57% and 91.70% of those who needed it.

To evaluate the prevalence of multiple dental caries and the nature of nutrition, dental examination and questioning of 163 children were conducted among which boys were 48.3% and girls – 51.7%.

Tooth involvement by caries process was evaluated on the basis of the following indicators: caries prevalence (in%), caries intensity (CPV – caries, filling, removed (refers to permanent teeth) and CPV + kp, CP – caries, filling, removed (related).

Determined the condition of the teeth using a standard dental tool kit – noted the presence of carious cavities and their localization, the presence of complicated caries, removed teeth, the condition of the fillings, secondary caries, the presence of dental deposits.

Figure 1 shows the results of the distribution of children according to the evaluation of oral hygiene

The data in Fig. 1 indicate that there was a mild plaque in all the children who were examined, but the level was different. The highest level of mild plaque was found in children aged 15 and 10 years, which was 90.0% and 73%, respectively. And the lowest level was found in children aged 11 and 12 years – 28.0% and 31.5% respectively.

Solid plaque was detected in children aged 12 and 15 years – 10.5% and 20.0%, respectively.

The next step in the study was to study the presence of temporary and permanent tooth decay in children. The obtained data are shown in Fig. 2.

The analysis given in Fig. 2. The data indicates that all of the children examined had permanent teeth affected by caries. The highest level of permanent tooth impression was observed in children aged 12 and 9 years: 50.0% and 40.0%, respectively, and the lowest in children 10 and 11 years – 18.0% and 19.0%, respectively.

Temporary teeth affected by caries in children 9 and 12 years: 10.0% and 10.5%, respectively.

The results of the study on the intensity of caries of temporary and permanent teeth in theexamined children are presented in Fig. 3.

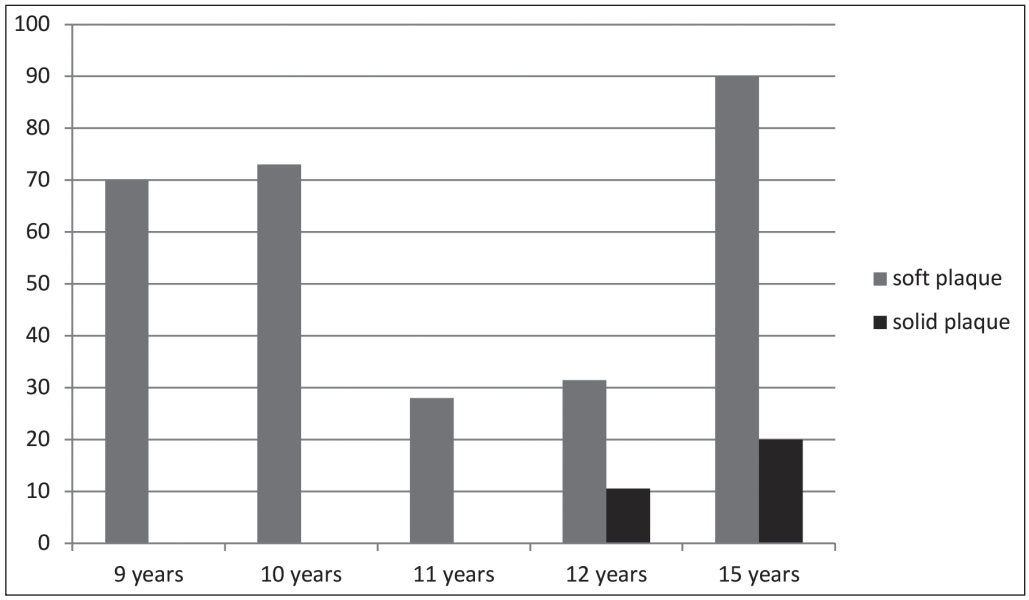

Fig. 1. The distribution of children byoralhygiene, %.

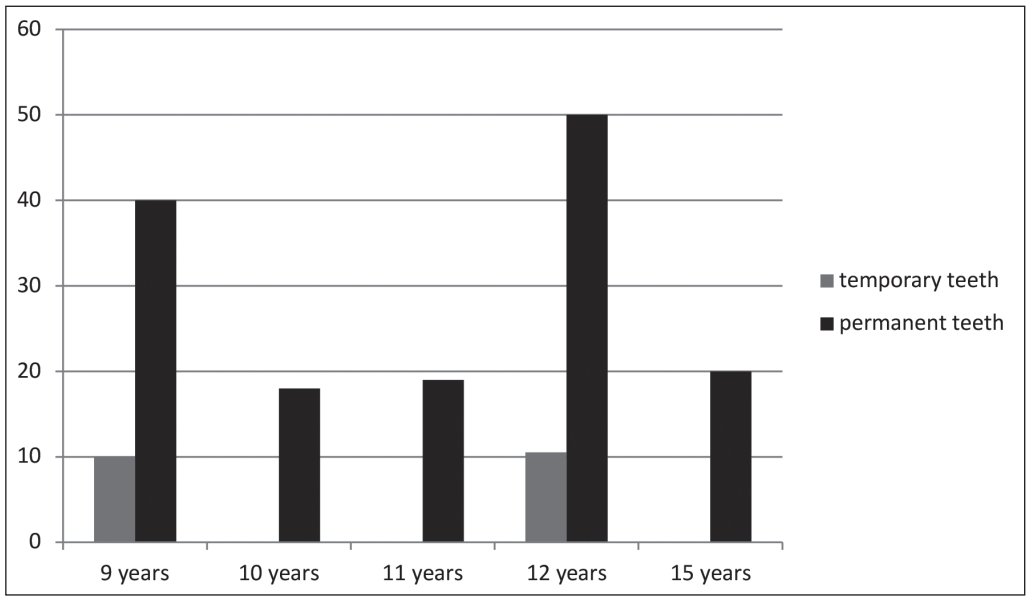

Fig. 2. Caries prevalence in examined children, %

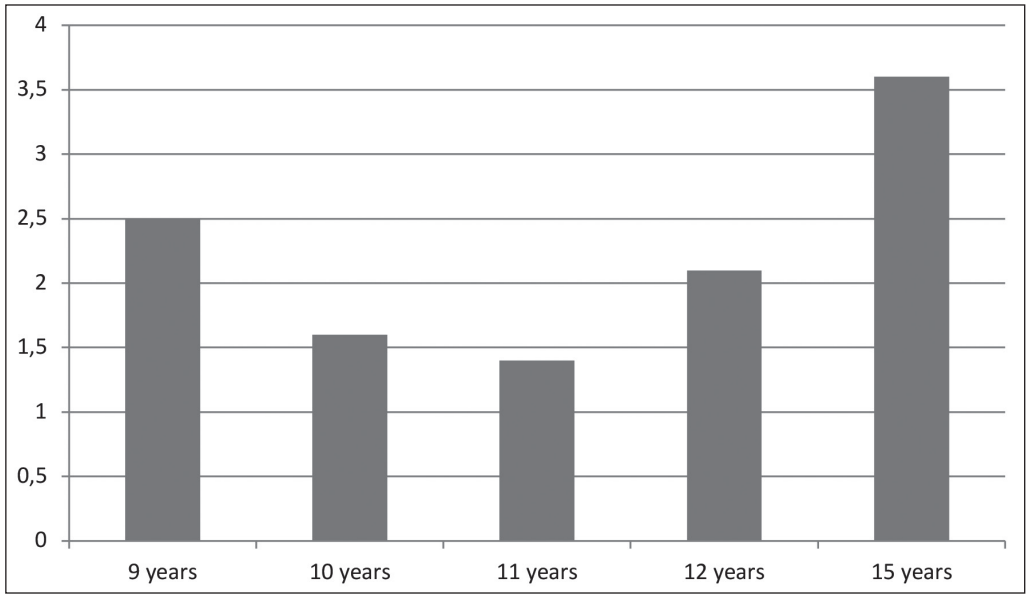

Fig. 3. The level of dental caries intensity in the examined children,%

Table I. Structureofdentalcariesintensityindexandprevalenceofcariescomplications of caries,%

Child'sage	Toothdecay	Sealedteeth	Teethremoved	Complicationsofcaries
9 years	40,0	70,0	-	-
10 years	54,5	64,0	-	9,1
11 years	66,0	66,0	9,0	9,0
12 years	79,0	31,5	-	24,0
15 years	60,0	70,0	10,0	-

According to the data in Fig. 3 it is seen that the average level of caries intensity, according to WHO criteria, was detected in children aged 15 years, which is 3.6%. Other children had a low level of caries intensity: 9 years – 2.5%, 10 years – 1.6%, 11 years – 1.4%, 12 years – 2.1%.An important step in the study was to study the structure of the dental caries intensity index and the prevalence of caries complications in the examined children. The results are shown in Table I

The results of the analysis are given in table I. And the data of the structure of the index of dental caries intensity and the prevalence of caries complications make it possible to state the following:

- caries affected teeth in all children with the highest level of age 12 (79.0%) and 11 (66.0%) and 15 (60.0%) years;
- Sealed teeth in children of all ages, examined with the highest level at the age of 9.15 (70.0% each) and 11 (66.0%) years;
- removed teeth found in children aged 11 and 15 years: 9.0% and 10.0%, respectively;
- complicated dental caries were detected in children aged 10-12 years with the highest level in children aged 12 years (24.0%).

We studied the location of dental lesions in children. Most of the affected teeth are found to be molars and premolars on both jaws. In the upper jaw, the tooth loss is 55% and 30%, respectively, in the lower jaw 75% and 25%.

In the clinical examination of children, an orthodontic index was also established as a pathology of the bite. Bite pathology was most pronounced in children 12 years of age (31.5%).

Considering that in the prevention of caries and pathology of the bite of great importance is the biological value of the daily diet and the nature of food we conducted a sociological study among the examined children in order to study their diet.

Based on the results of the survey of the surveyed children, it was found that in the daily diet of children, regardless of age and sex in which tooth decay is detected, there is a fairly uniform set of foods. It has been established that children have low consumption of milk and lactic acid products, which can cause a decrease in the amount of calcium and phosphorus in the body. It is revealed that daily milk and lactic acid products are included in the diet of children aged 9 years at 45.0%, 10-12 years – 26.0%, 15 years – 35.0%.

The survey results show that only 45.0% of respondents consume fresh fruits and 67.0% fresh vegetables daily. This is especially true of most children of 12 and 15 years with multiple caries. If at the age of 11 44.0% of children with multiple caries eat fruits daily, then at the age of 15, the percentage of such children decreases to 32.5% of children. At that time, 56.3% of children with caries consume sweets and pastries daily, as well as more than half (53.1%) of children with multiple dental caries consume sweet sodas every day, especially between the ages of 10-12.. At the age of 10, 67.0% of the boys use these drinks regularly. Only 22.1% of children with multiple caries rarely consume sugary drinks and never only 19.9% of those polled. More than half of children with multiple caries consume candy every day (65.7%). Sweet tea is consumed by 67.7% of children with caries.

It was found that over 80% of the respondents applied for dental care regardless of their age. At the same time, at the age of 10 and at the age of 15, more girls appealed, and at the age of 10 -12 years – most boys.

Children aged 9-10 years 7-8 times a day consume cariesogenic products. And boys consume fruits more often than girls, as well as foods rich in carbohydrates and starch. Girls consume sweet dairy products more often than boys during meals. It has been established that the thirst quenching in children is mainly caused by the use of sweetened drinks, juices and sweet carbonated drinks.

CONCLUSIONS

1. Analysis of statistical data showed that for the period 2009-2018 in Uzhgorod the number of children covered by dental preventive examinations decreased by 28.9% and amounted to 13706 children, which is 60.22% of their total number. During this period, the proportion of children requiring rehabilitation decreased by 37.1% to 459.35 per thousand inspected. Remediation of the oral cavity was carried out 91.7% of those who needed it.
2. On clinical examination, 163 children aged 9 to 15 years had a mild plaque found in all children with the highest level (90.0%) in children aged 15 years and a solid plaque detected at the age of 12 and 15 years – 10 , 5% and 20.0% respectively. The highest level of permanent tooth impression was found in children aged 12 and 9 years: 50.0% and 40.0%, respectively, and the lowest in children 10 and 11 years – 18.0% and 19.0%, respectively. Temporary teeth are affected by caries in children 9 and 12 years: 10.0% and 10.5%, respectively. Average caries intensity, by WHO criteria, was found in children aged 15 years, which is 3.6% The rest of children have a low level of caries intensity: 9 years – 2.5%, 10 years – 1.6%, 11 years – 1,4%, 12 years – 2,1%.

3. When studying the structure of the index of dental caries intensity and the prevalence of caries complications it was found that caries affected teeth in all children with the highest age of 12 (79.0%) and 11 (66.0%) and 15 (60.0%). years; dental fillings in children of all ages, examined with the highest level at the age of 9.15 (70.0% each) and 11 (66.0%) years; removed teeth found in children aged 11 and 15 years: 9.0% and 10.0%, respectively; complicated dental caries were detected in children aged 10-12 years with the highest level in children aged 12 years (24.0%). The study found that the upper jaw of the molars and premolars was respectively 55% and 30%, the lower jaw 75% and 25%.
4. The results of the sociological survey revealed that, regardless of age and sex, children who have caries revealed that they do not eat rationally: they consume little milk and lactic acid products (26.0% -45%), 45.0% daily eat fresh fruits and 67.0% fresh vegetables, most consume sweets and sugary drinks daily.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

STUDY OF PATHOGENIC FACTORS OF *E.COLI* ISOLATED FROM PATIENTS WITH PERITONITIS

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ABSTRACT

The aim: To study the biological properties of museum and clinical strains of *E.coli* isolated from patients with peritonitis.

Materials and methods: It was used 94 strains (clinical, museum and reference). The ability of *E. coli* to adhere was investigated by hemadhezive method to formal human erythrocytes of 0 (I) Rh-positive blood group. The study measured the ability of microorganisms to produce gelatinaze, caseinase, fibrinolysin, hemolysin. To control of the enzyme activity the positive and negative control with reference strains were used. Synchronisation of cultures activity before experiments was achieved by one-time effect of low temperature (+4 C) during 30 minutes.

Results: To investigate the pathogenic factors of *E. coli* we carried out determining of proteolytic, gelatinous, caseinous, fibrinolytic, haemolytic and adhesion properties.

Conclusions: In our investigation pathogen icspecies of Esherihies are virtually indistinguishable from representatives of normal microflora on its morphological, biochemical and cultural properties. During investigation of serological properties of selected of *E. coli* strains (n = 94) 65.8% of pathogenic serotypes were revealed. Moreover, all marked *E. coli* isolated from the abdominal cavity of children and adults, as well as museum strains related to enteropathogenic E. coli (0127: K63, 033: K-) and 1 – to enteroinvazive *Escherichia coli* (0144: K-).

KEY WORDS: *E.coli*, peritonitis, biological properties

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INTRODUCTION

The human's colon maintains a microbial density approaching 10^{12} organisms per gram of feces, representing a perfectly balanced ecosystem. The commensal microbiota, derived from the Latin commensal and meant sharing a table, consists of more than 400 species and lives in perfect harmony with the human intestine [1,2].

Escherichia coli is a commensal inhabitant of the intestinal tracts of healthy humans and many animal species, but it can also cause a wide range of diseases, ranging from diarrhea to extraintestinal infections [2, 3]. But *E. coli* is more than just a laboratory workhorse or harmless intestinal inhabitant; it can also be a highly versatile, and frequently deadly, pathogen. Extraintestinal pathogenic *Escherichia coli* (ExPEC), the specialized strains of *E. coli* that cause most extraintestinal *E. coli* infections, represents a major but little-appreciated health threat. Although the reasons for their evolution remain mysterious, by virtue of their numerous virulence traits ExPEC clearly possess a unique ability to cause disease outside the host intestinal tract. Broader appreciation of the existence and importance of ExPEC and better understandings of their distinctive virulence mechanisms, reservoirs, and transmission pathways may lead to effective preventive interventions against the morbid and costly infections ExPEC cause [4,5].

Escherichia coli is one of the most common isolates in clinical microbiology laboratories and classified into three major groups: commensal strains, intestinal pathogenic strains, and extraintestinal pathogenic strains, according to their biological significance to humans [5, 6, 7]. Purulent peritonitis in modern conditions differs from the bacteriological point of view of a combined flora with ever-increasing pathogenic properties. At the same time, *Escherichia coli* retains the dominant role in the microbial etiology of peritonitis [8].

As pathogenic factors we define adaptive mechanisms of infectious diseases. First of all you should decide on such a fundamental property of bacteria as pathogenic.

The presence of microbial pathogenicity factors is important for assessing the etiological significance.

For *E. coli*, pathogenicity is not a species sign and is not related to a specific serogroup. They are able to realize their pathogenic potential and cause disorders in the human body, limited only by those genetic determinants that have a specific strain of *E. coli*.

THE AIM

To study the biological properties of museum and clinical strains of *E. coli* isolated from patients with peritonitis.

MATERIALS AND METHODS

It was used 94 strains (clinical, museum's and reference). The ability of *E. coli* to adhesion was tested with hemadhezive method on formalinized human erythrocytes of 0 (I) Rh-positive blood group [9].

Within the research we measured the ability of microorganisms to produce gelatinase, caseinase, fibrinolysin, hemolysin.

Positive and negative control with reference strains were used to control of the enzyme activity.

Synchronisation of cultures activity before experiments was achieved with simultaneous time effect of low temperature (+4 C) within 30 minutes.

RESULTS AND DISCUSSION

To investigate the pathogenic factors of *E. coli* we carried out proteolytic, gelatinous, caseinous, fibrinolytic activities, as well as adhesion properties.

It was found that all investigated strains of *E. coli*, 94 in number, had proteolytic activity. This high degree of proteolytic activity wasn't found. All clinical and museum strains were weak active.

In addition to discussed above biological properties of obtained bacteria, the fibrinolytic activity was determined. This factor of pathogenicity aimed at dissolving fibrin, that limits local inflammatory center, and thus contributes to the generalization of the pathological process.

In the study on fibrinolytic activity of all clinical strains and isolated from relatively healthy individuals had a negative result. This statement corresponds to the date given in scientific literature due to which the strains of *E. coli* do not have the capacity for fibrinolytic activity.

In the study of pathogenicity factors the great importance is given to hemolytic activity. It is connected with cytotoxicity, here with the lysis direction, which action is not limited only on erythrocytes, but it also spreads on other cell types. The ability of many strains of *E. coli* to cause haemolysis of red blood cells of humans and animals is connected with the presence of α-hemolysin gene in cells that is a major factor of *E. coli* pathogenicity. Hemolytic active is shown up with thermally labile toxins, extracellular β-hemolysin and enterohemolizyn that localizes in the outer membrane of the bacterial cell and cytolizyn A. They "drill" plazmalema of red blood cells as well as other cells including phagocytes by making them viable.

It is known from the literature that *E. coli* strains with plasmid extraintestinal localization have α – hemolysin [10,11,12]. Produced cytotoxin strains are resistant to chemotherapeutic medicines and they are particularly dangerous [13].

We studied the hemolytic activity in *E. coli* strains extracted from children and adult patients with peritonitis, and also museum and strains that was isolated from relatively healthy people (group).

During analyzing the results of the determination of the hemolytic activity in deleted strains *E. coli* (n = 94) it was found that 4 (4,2 ± 2,0)% strains isolated from adults were able to produce α – hemolysin.

In all the clinical strains of *E. coli* being extracted from children and adults, as well as museum and isolated from relatively healthy people we found ability to gelatinase activity. It was established that in 4 (16,0 ± 7,3)% of strains, that was removed from adults, gelatinase activity was sharply positive with a positive result – 10 (40,0 ± 9,7)%. Also weak active strains- 5 (20,0 ± 8,0)% were found. We got a negative result in 6 (24,0 ± 8,5)% strains .

In the study of the museum strains of *E. coli* it was found that 2 (10,5 ± 7,0)% strains were with high positive activity of gelatinase – 13 (68,4 ± 10,6)% strains. There were strains with weak activity of gelatinase 4 (21,0 ± 9,3)% and those which had a negative result – 1 (5,2 ± 5,0)% strain. It should be noted that strains of *E. coli* extracted from children didn't have little and sharply positive gelatinase activity, but there were 2 (8,0 ± 5,4)% cases in which strains gave positive results in 48 hours, indicating a weak gelatinase activity. The vast number of strains were made up with *E. coli*, which had a positive activity gelatinase 22 (88,0 ± 6,4)%. Among the studied strains 1 (4,0 ± 3,9)% was with negative result.

During analyzing gelatinase activity in strains extracted from relatively healthy people, positive strains were 2 (8,0 ± 5,4)%, and weakly positive – 1 (4,0 ± 3,9)%. However, the interesting thing was that negative strains and those that gave positive results in 48 hours turned out the same number of 10 (40,0 ± 9,7)%.

The value of adhesive characteristics of bacteria for a microorganism could be considered from two positions. On the one hand, the adhesive capacity of indigen microflora is one of the factors in the realization of resistance, the intestinal mucosa colonization and obstacles for attachment to receptors of the mucosal pathogens. On the other hand, the development of dysbiotic violations of adhesive properties of opportunistic microflora is considered as a pathogenicity factor because they allow microbes to get fixed on the surface of the skin or mucous membranes and colonize this biotype by reaching a certain level of population. Now it is shown the adhesive nature of the changes of microbes characteristics in development of microecological disorders of oropharynx and nasapharynx, vagina; proposed approaches to lowering of the adhesion of conditional – pathogenic bacteria were proposed. At the same time, data on the biological properties of intestinal microbiota representatives were quite a few that is probably due to species diversity of biotope.

From the literary sources it is known that adhesion plays an important role in the development of infection, it also determines its beginning and promotes the further invasion of microorganisms in the host tissue. Pathogenic potential of microorganisms, when translocated from the place of natural habitation to other biotopes significantly increases. We have studied the adhesive activity of strains of *E. coli* extracted from children and adult patients with peritonitis, and museum strains isolated from relatively healthy people. The results of the study of adhesive properties *E. coli*, extracted from patients with peritonitis, museum strains and strains isolated from relatively healthy people are shown in Table. I.

Table I. Level adhesivity of strains of E.coli isolated from the patients with peritonitis, museum strains and strains isolated from relatively healthy people (group)

Indices of adhesion	E.coli strains isolated from children (n = 25), M ± m	E.coli strains isolated from adults (n = 25), M ± m	Museum strains of E.coli (n = 19), M ± m	E.coli strains are isolated from conventionally healthy people (comparison group) (n = 25), M ± m
Average adhesion	3,3±0,3	3,2±0,3	1,9±0,2	2,5±0,2
Adhesion coefficient (%) (C)	41,1±9,8	35,4±9,6	30,0±10,5	28,3±9,0
Adhesion index	4,8±0,4*	4,6±0,3	2,7±0,3	3,3±0,3*

Note: the difference is statistically significant parameters (p <0.001)

Table II. Characteristics of enzymatic activity of strains of E. coli, isolated from children and the adult patients with peritonitis, museums and strains isolated from healthy conventionally (comparison group) (n = 94)

Test or substrate	Percentage of positive strains,%
Citratus of Simons	0
Glucose (A,G)	100
Hydrogen sulfide	0
Indole	91,5
Lactose (A)	90,4
Lysinedecarboxylase	98
Mobility	89,4
Ornithinedecarboxylase	87,2
Reaction with methyl red	100
Sorbitol (A)	89,4
Urea	0
Voges – Proskauer test	0

Note. AG - acid and gas, A - acid.

The study found that the average number of red blood cells, which were involved in the process of adhesion (C) of *E. coli* strains extracted from adult patients with peritonitis, museum strains and strains isolated from relatively healthy people (group) did not differ significantly (p> 0.05).

During analyzing the results of adhesive properties of *E. coli* strains isolated from adult patients with peritonitis the following results were obtained: 5 strains had high adhesive activity corresponding to (20,0 ± 4,4)%, average – 20 (80.0 ± 8,0)% of 25 studied strains. For comparison group strains (strains conventionally extracted from the intestine of healthy people), the average was adhesiveness in 2 (8,0 ± 2,8)% of cases, the proportion of strains of low-adhesive *E. coli* of this group was 23 (92,0 ± 8,4) %.

In the study of strains of *E. coli* isolated from children suffering of peritonitis we can say that strains with secondary and high adhesiveness were turned out 12 (48,0 ± 6,5)% and 13 (52 ± 6,7)% in accordance.

Data analysis of the study of adhesiveness museum strains of *E. coli*, showed that 5 strains (26,3 ± 5,0)% were with high adhesiveness and 15 of 19 strains (78,9 ± 8,2)% were with middle adhesiveness.

Thus, all studied of strains of *E. coli* had adhesive properties. Higher rates of adhesion of E.coli strains isolated from children and adult patients with peritonitis, in comparison with strains isolated from conditionally healthy people. Having strains with high adhesiveness may indicate that inflammation increases.

In the analysis of the obtained results it could be concluded that the selected strains are not highly active according to all criteria.

Of course, not-specific method cannot guarantee the final determination of the role of the selected strain as the causative agent of purulent – inflammatory processes, including peritonitis. Only the set of objective indicators, which include sensitivity to antibiotics and can determine the proportion of a particular strain of infection.

However pathogenicity factors would examine the cultural, morphological, and biochemical properties tinctorial 94 strains of *E. coli* extracted from children and adult patients with peritonitis and museum strains. The results that were obtained in the study of biological properties that made it possible to assign them to form *E. coli*.

The first step in the studying of the nature of the growth of *E. coli* on Endo medium. Colonies were evaluated with the following characteristics (size, shape, lifting above the surface, features of the edges, density, texture). Equally important is the color of the isolated colonies of *E. coli* on Endo medium that depends on differentiating components of the environment.

Because of (18 – 24) hours of incubation, the medium Endo, *E. coli* colonies had the correct round shape, smooth,

convex surface, smooth edge. The red colonies with a metallic shine on the nutritious medium were lactose positive colony. Pink or colorless colonies with a reddish center lackluster – strains which are poorly fermentated by lactose. Remained colorless colonies were not lactose fermentated.

To confirm the bacteria belonging to the family of products determined Eterobacteriaceae enzymes catalase and oxidase, subplanted colony on the combined environment for primary identification (Kligler Iron Agar).

The feedback list of the growth on the agar Kligler Iron Agar was carried through (18-24) h incubation, herewith noted the presence of gas generation, hydrogen sulphide production, fermentation of lactose and glucose. Fermentation of glucose and lactose was determined to change the colour of the environment in the column and the beveled part. After the second phase of identifying those selected crops that fermented acids and glucose to only gas or acid fermented or not fermented lactose and no hydrogen sulfide produced.

To determine the biochemical properties isolated strains took a minimum number of differentiating tests (Table. II).

One of the "key" biochemical tests for the identification of bacteria of the genus *Escherichia* is the ability to dispose of sodium acetate. But there are «inactive» *E. coli*, which gives a negative result in the majority of use tests, making them similar to *Shigella* and hafnium. Therefore, it is appropriate to determine the biochemical properties in the fullest possible extend but not only to evaluate single key tests.

Depending on the presence of these or other pathogenicity factors, serological markers and epidemiological features, diarrhea *E. coli* are divided into the following categories: enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterohaemorrhagic (EHEC) And enteroaggregate or enteroadergic *E. coli* (enteroaggregative, enteroadherent – EaggEC, EAEC) [2,3].

With a significant increase in the *E. coli* concentration in the intestinal biotype, the translocation of microorganisms into other organs and systems is possible, with such changes the coliform biotope can serve as an endogenous source of high – valued clones of conditionally pathogenic microorganisms capable of reducing the colonization resistance of individual biotopes[1]. Among the infections of the extracurricular localization that can cause the colon, distinguish the infection of the urinary and respiratory tract, intra-abdominal, skin and soft tissues, as well as generalized [2,3].

In our study, the pathogenic species of the esherichia do not actually differ from the representatives of the normal microflora according to their morphological, biochemical and cultural properties. Therefore, it was necessary to carry out serological diagnostics for the identification of pathogenic serotypes. The presence in the pathological material of *E. coli* of a certain OK group was determined with a positive result of the agglutination reaction of live cultures with the corresponding monovalent OK-serum, as well as the proven cultures – with adsorbed O-serum.

In the study of serological properties of selected strains of *E. coli* (n = 94), 65.8% of pathogenic serotypes were detected. At the same time, all isolated *E. coli* isolated from the abdominal cavity in children and adults, as well as museum strains belonged to the EPCP (O127: K63, O33: K-) and 1 to EICP (O144: K-).

CONCLUSIONS

In our investigation pathogenic pieces of Esherihies are virtually in distinguish able from representatives of normal microflora on its morphological, biochemical and cultural properties. During investigation of serological properties of selected of *E.coli* strains (n = 94) 65.8% of pathogenic serotypes were revealed. Moreover, all marked *E.coli* isolated from the abdominal cavity of children and adults, as well as museum strains related to enteropathogenic *E. coli* (O127: K63, O33: K-) and 1 –to enteroinvazive *Escherichia coli* (O144: K-).

Abbreviations:
ExPEC – Extraintestinal pathogenic *Escherichia coli*,
E.coli – Escherichia coli,
A – acid, G – gas.

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ORIGINAL ARTICLE
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ROLE AND PLACE OF ULTRASONOGRAPHY IN DIAGNOSTICS OF ADHESIVE INTESTINAL OBSTRUCTION

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ABSTRACT

The aim: To study the possibilities of ultrasound in adhesive ileus obstruction diagnosis and indications for surgical treatment determination.

Materials and methods: The results of diagnosis and treatment of 60 patients with acute adhesive intestinal obstruction with comparative use of X-ray and ultrasound methods were analyzed.

Results: The use of a standard X-ray examination of the abdominal organs upon admission to the hospital allowed the diagnosis of acute adhesions of small bowel obstruction in only 32 (53.3%) patients. Transabdominal ultrasonography allowed the establishment of adhesive ileus during hospitalization in 53 (88.3%) patients and also in 7 (11.7%) during a dynamic examination 2-3 hours after admission. Due to the lack of positive effect from the conducted conservative therapy relying on the data of ultrasound studies, 33 (55%) patients were operated on. During X-ray examination, only 10 (30.3%) of these patients demonstrated negative X-ray dynamics, and with an ultrasound study, deterioration of echo pictures was observed in all 33 (100%) patients.

Conclusions: Compared with the X-ray method, transabdominal ultrasonography is established to be safe, affordable, simpler, non-invasive and highly informative method for the study of acute adhesions of the small intestinal obstruction. The method allows the assessment of the ongoing conservative therapy dynamics and determination for surgical treatment indications.

KEY WORDS: acute adhesive small-intestine obstruction, diagnostics, X-ray, ultrasonography

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INTRODUCTION

Acute adhesive small-intestine obstruction (AASIO) still remains complicated and not fully resolved surgical problem. Its relevance primarily depends on the growing number of various planned and urgent abdominal operations complicated by AASIO progress in up to 20% of cases as well as high percentage of post-operative complications (8-31.4%) and mortality up to 25% [1, 2, 3, 4, 5].

High AASIO recurrence rate up to 30-69% with further open adhesiolysis performance with post-operative mortality at the level of 55% adds relevance to the problem. [6, 7, 8, 9].

AASIO in accordance with its pathogenic origin differs from classical mechanical ileus and is inherently a mixed form of small-intestine obstruction. Its share in the general structure of acquired acute intestinal obstruction (AIO) according to summary data of some authors comes near 50% [1, 3, 10].

AASIO diagnostic algorithm is not standardized, so high percentage of diagnostic errors (16-34%) remains even in conditions of hospital for in-patients, X-ray method of ileus diagnostics still remains prevalent [8, 9, 11].

X-ray computer tomography (CT) able to diagnose AASIO with high level of accuracy and carry out differential diagnostics with other types of intestinal obstruction has recently been widely publicized. Though CT is more often used as additional method of the study with ambiguous interpretation of the data of standard instrumental diagnostics and not always can be used because of its costliness and limited availability [11, 12].

Unfortunately such simple, safe and highly informative and affordable method of AASIO diagnostics as ultrasound study (USS) still finds no wide practical application [1], its application will allow the improvement of diagnostics results and adhesive ileus treatment.

THE AIM

The aim of the study is to investigate the abilities of USS method in adhesive ileus diagnostics and surgical treatment determination.

MATERIALS AND METHODS

The work is based on the analysis of diagnostics results and surgical treatment of 60 patients with AASIO being treated in the surgical department of Kharkov CCGH №25 and PHF "Valkovskaya CRH" in 2015-2018.

Men were 27 (45 %), women – 32 (55 %). The age of the patients varied from 18 to 78 years old, average age comprised 62±12. Post-operative ventral hernia was found in 7 (11.7%) patients from them, umbilical hernia – in 2 (3.3 %), history of abdominal trauma – in 1 (1.7 %).

Besides standard physical studies diagnostic algorithm included history taking with data refinement as for pres-

ence and character of previously conducted operations, inflammatory abdominal diseases and abdominal trauma.

Instrumental stage of the investigation included abdominal organs (AO) X-ray study upon admission and in treatment dynamics (from 2 to 6 hours) after giving contrast. In the diagnostics of AIO intestinal pneumatosis presence, Kloiber bowls and small-intestine "arches", small intestine (SI) "cross striation" symptom were taken into account.

Regardless X-ray study AO USS was done upon admission and in conservative treatment dynamics in "real time" with the use of 3.5 – 5 MHz convex sensor. USS was done without initial preparation of the patients in lying and standing position. The intestine was studied poly-positionally and poly-projectionally with the search of optimal "ultrasound window". Revealing of extended and angulated SI loops, intraluminal deposition of liquid and flatus, pendulum chyme movement presence, restriction of mobility of peritoneal sheet and CT at forced "belly breathing" were considered echo-sonographic symptoms of AASIO. The thickness of intestine wall, the height of Kerkring folds and the distance between them were also measured and compared in dynamics, peristalsis depth and frequency of SI loops were studied. Free abdominal liquid presence, stomach contents congestion and pathologic foci (tumors, foreign bodies, etc.) were determined.

The results of the study were statistically processed with the use of standard Microsoft Excel 2010 program package.

RESULTS AND DISCUSSION

At hospitalization the diagnosis of AASIO was clear in 32 (53.3%) patients, in 22 (36.7%) cases the signs were moderately pronounced and in 6 (10 %) – poorly expressed or absent.

According to the history data in 55 (91.7%) patients AO and retroperitoneal operations had been previously performed. In 12 (20%) of them cavity operations were done twice and more, 6 (10 %) patients were operated on AASIO earlier.

It was stated that in 5 (8.3%) patients AO operations were not earlier performed. In 2 (4.2%) cases incorrigible umbilical hernia was diagnosed. In 2 (3.3%) more patients a history abdominal trauma was found. 1 (1.7%) patient earlier received conservative treatment for appendicular infiltrate without further appendectomy.

At X-ray study upon admission to the hospital AIO was confirmed only in 32 (53.3 %) patients with pronounced and moderate clinics. Multiple Kloiber bowls and small intestine "arches" were found in them, in 8 (13.3%) cases cross striation of SI was found. In remaining 28 (46.7%) cases patients manifested no pathology or only local SI pneumatosis were found (fig.1).

The conducted transabdominal USS to the patients upon admission to the hospital allowed determination of ASSIO in 53 (88.3 %) patients. In 7 (11.7%) more cases the ultrasound picture of AIO was found at dynamic USS in 2-3 hours after initial examination. Echographically in all 60 (100%) patients the phenomenon of "fluid sequestration" into the intestine lumen and hyperpneumatosis of its leading gut were found (fig.2). The diameter of extended SI loops, filled with liquid varied from 2.6 to 4.4 cm and comprised in average 3.2 cm ± 0.3 cm. Back-and-force SI chyme motions were found in 58 (96.7%) patients, and its wall thickening comprised 0.4 cm ± 0.01 cm. Intraluminal content of SI allowed the visualization of its mucosal folds (fig.3). Kerkring folds of the greatest size were found in its primary tracts, their height was 1.1 cm ± 0.02 cm, and the distance between them was 1.1cm ± 0.01 cm.

The level of obstruction was judged about the data of SI loops localization in abdomen as well as clarity of mucosal wrinkles which height and number decreased sufficiently in its distant region.

The conducted USS allowed the determination of free from adhesive process abdominal regions as well as various degree of SI loops fixation clarity by adhesions between themselves and to parietal peritoneum of the anterior abdominal wall. Mobility of SI loops regarding anterior abdominal wall at forced belly breathing of the patient was decreased and comprised 1.4 ± 0.6 cm (in remote zones it was 3,7 ± 0,7 cm).

In 8 (13.3%) patients at primary USS and in 10 (16.6%) more at repeated study interloop (fig 3) and free liquid were found in various abdominal areas in the amount from 200 to 1000 ml, which in no case was determined on X-ray. The given ultrasound sign is the objective criteria of the negative dynamics of the disease course and an indication to urgent surgery.

In 27 (45 %) patients after conducted conservative treatment the improvement of clinic-instrumental picture was marked. On X-ray the number of Kloiber bowls and small intestine "arches" disappeared or decreased in these patients, intestine pneumatosis regressed, in 6-12 hours the contrast was found in colon.

In its turn with echosonography in the same patients positive ultrasound dynamics was marked in 2-3 hours after conservative therapy. Reducing of the width of SI lumen, Kerkring folds height were found, chyme motions became progressive which proved about SI passability.

In 33 (55 %) patients positive effect from conducted conservative therapy was not marked and they were later operated on. On X-ray only in 10 (30.3 %) from these patients negative X-ray dynamics was marked with Kloiber bowls number and size growth, appearance of small intestine "arches" and SI cross striation. Absence of contrast in colon and presence of contrast depot in SI (fig 4) predetermined indications to operative treatment of these patients.

On USS worsening of echo picture was observed in all 33 (100 %) patients. SI diameter increased in 3.4 cm ± 0.1 cm, chyme stratification was found in it. Kerkring folds height and distance between them increased up to 1.2cm ± 0.1cm and 1.3cm ± 0.1cm, consequently. Slowing down of back-and-forth motions of chyme was marked which proved about SI peristalsis oppression.

On X-ray dynamic study of contrast passage vomiting and stomach drains presence presented complications in objective assessment of its progress after oral intake. The

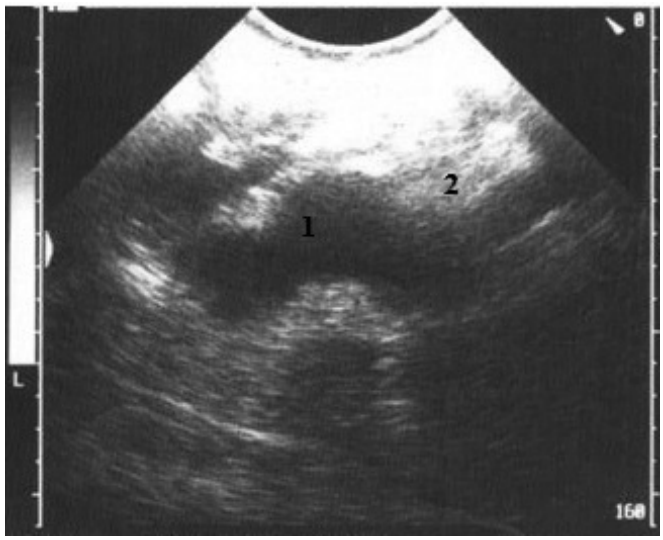

Fig. 1. X-ray of the patient A, after 3 hours from the onset of the disease. Only small intestine pneumatosis is determined (1)

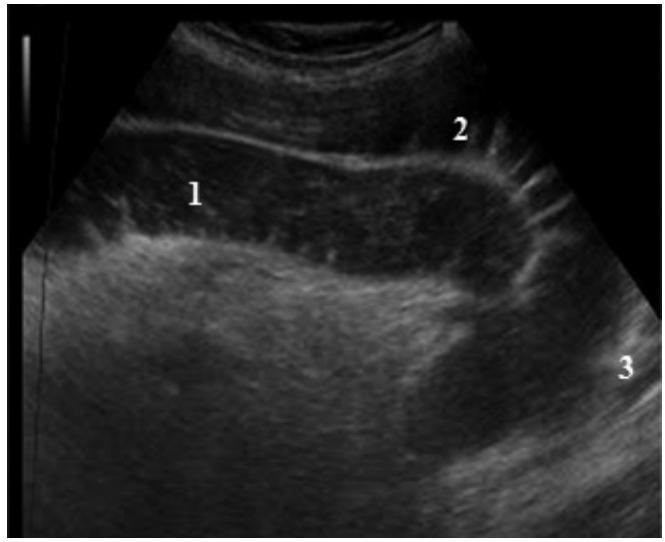

Fig. 3. Patient B. Echogram of the left iliac region. ASSIO, 6 hours from the onset of the disease: ileum lumen dilation with fluid deposition (1), lengthening and thickening of Kerkring folds (2), abdominal free liquid (3)

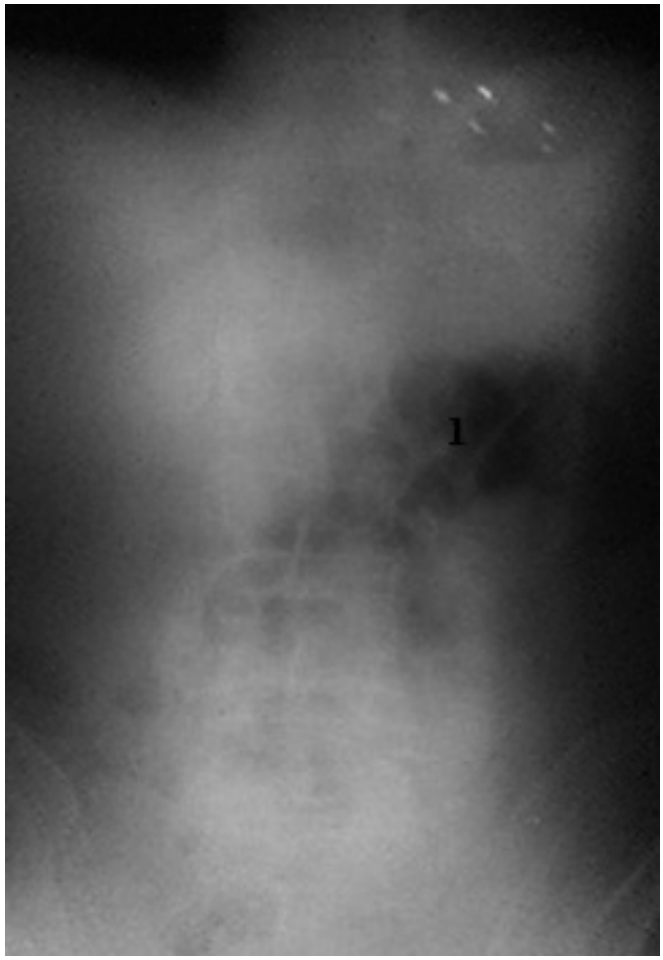

Fig. 2. Echogram of the patient A. High AASIO, 3 hours from the onset of the disease. SI lumen dilation with fluid (1) and flatus (2) deposition is determined

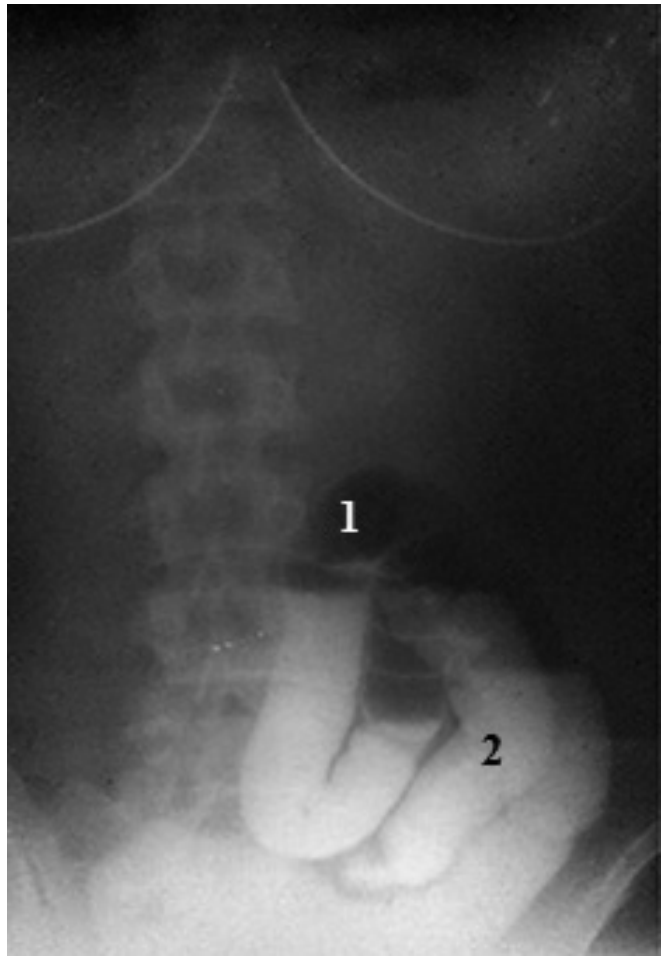

Fig. 4. X-ray of the patient B. After 3 hours after contrast giving. Flatus (1) and contrast depot in ileum (2) are determined

lack of methodology is later in comparison with USS assessment of the results (in 6 hours and more) and necessity to perform multiple X-rays.

33 (55 %) patients in all were operated on. 8 (13.3%) patients were operated urgently after preoperative preparation and 25 (41.7%) more were operated on as the result

of inefficiency of conservative therapy. Various options of adhesiolysis were conducted to the patients, SI resection was done in 3 (9.1%) cases. Besides adhesiolysis allodherinoplasty was conducted in 2 (6.1%) patients, cholecystectomy about cholelithiasis – in 3(9.1%) cases and appendectomy – in 1 (3.0%) patient in combination.

Thus basing on a number of works of domestic and foreign authors [2, 3, 4, 7, 8, 10] so far the X-ray ASSIO diagnostics remains the primary method which is inherently more expensive, difficult to perform in serious patients and as a rule less informative upon admission of the patient to the hospital than USS. In its turn CT is highly informative, but costly method of ASSIO diagnostics. From our point of view CT should not be used during ASSIO routine diagnostics, but only with ambiguous interpretation of traditional diagnostics methods.

Our study allows the approach to adhesive ileus diagnostics from new positions, and the worked out program with USS priority use allows the statement of detailed diagnosis in short terms, the definition of the efficiency of the conservative treatment by several objective criteria. More than that, the value of the program is in getting pathognomonic preoperative data allowing the definition of clear indications to urgent surgical treatment. Correct interpretation of the received echographic data allows the definition of localization, extent, prevalence of the adhesive process and particularly involvement of parietal peritoneum of the anterior abdominal wall into it, which is important when undertaking atraumatic "rational" operative access to abdominal organs. The received results testify in favor of more wide and routine use of USS method in ASSIO diagnostics due to its accessibility, safety and high informativeness.

CONCLUTIONS

1. Comparative study of X–ray and USS methods efficiency allowed to state, that ultrasonography sensitivity during ASSIO initial diagnostics approaches 100%, while X-ray comprises 53.3 %.
2. Transabdominal ultrasonography is an invasive, accessible and highly informative method of ASSIO diagnostics, having a number of advantages in comparison with X-ray method, being safer, more simple and possible to be used even with seriously ill patients.
3. Wide implementation of ultrasonography in the AIO diagnostics will allow the improvement the examination results and, respectively, treatment of this category of patients.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

GENETIC POLYMORPHISM IN PATIENTS WITH EARLY AND LATE ONSET OF ULCERATIVE COLITIS

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ABSTRACT

The aim was to investigate SNPs of TLR-2,3,4, NOD2/CARD15, JAK-2, and IL-10 in patients with the early and late UC onset.

Matherials and methods: 126 patients with UC were investigated. To assess the predisposition of the early and late UC onset the incidence of the following SNPs: *Arg753Gln TLR2* gene, *Phe412Leu* TLR3 gene, *Asp299Gly* and *Thr399Ile TLR4* gene, *C-819T, G-1082A* and *C-592A* gene *IL-10*, *Val617Phe* gene JAK2, *Gly908Arg* gene NOD2/CARD15 were analyzed.

Results: 76 patients had early disease onset and 50 had a late one. SNPs of TLR3 were observed in 50.8% cases. TLR4 polymorphism was more common than TLR3, and was observed in 81 (64.3%) UC patients. Polymorphism of NOD2/CARD15 and IL-10 genes were revealed with almost the same frequency 49 (38.9%) and 50 (39.9%) patients, respectively.

Conclusions: Polymorphisms of TLR-2,3 genes and TLR4 Asp299Gly, NOD2/CARD15 prevailed in patients with the late UC onset that allows to suppose that bacterial flora plays one of the key roles in modification of immune response and UC development. In patients with early UC onset polymorphisms of the JAK2 and IL-10 genes prevailed responsible for the cytokine cascade activation and cause the immune mechanism that might lead to a more aggressive course of the disease.

KEY WORDS: ulcerative colitis; TLR-2,3,4; NOD2/CARD15; JAK 2; IL-10

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INTRODUCTION

Genetic analysis acquires an important role and might be pivotal in diagnosis, prevention and treatment of inflammatory bowel disease (IBD) in the nearest future [1, 2, 3]. Despite the upward trend in the incidence of ulcerative colitis (UC) at a young age, in recent years there has been revealed an increase number of patients whose UC onset happened in the second half of life (after 50 years). At a young age (20-35 years old), the peak of the incidence of UC is at the age of 20-22 years. The second peak of the disease is detected twice as often as the first, and it is diagnosed in elderly age of 50-55 years (late UC onset). The development of UC in patients with late onset is associated with the accumulation of predisposing and provoking factors (genetic predisposition, unfavorable environmental factors, eating disorders, allergization, medication[1, 2, 3, 4, 5] and determines the clinical features of UC: a greater hospitalization rate, an early need for steroid and immunosuppressive drugs compared with a group of young patients. The age-related features of the incidence of UC are associated with various factors (gender, place of residence, nationality, etc.), where genetic predisposition has the leading role [6, 7].

The predisposition to the development of UC is determined by the polymorphism of individual genes. NOD2/CARD15 assets nucleotide factor NF-κB, and induces transcription of tumor necrosis factor-α. The main role in genetic susceptibility to the violation of bacterial colonization is played by toll-like receptors (TLR). It is possible that TLR polymorphism in the intestine contributes to the formation of different types of response to bacterial antigens and affects the composition of the intestinal microbiota. Interleukin-10 (IL-10) is a key regulator of the immune response, the main anti-inflammatory cytokine. A correlation of single nucleotide gene polymorphisms (SNPs) in the promoter region of the IL-10 gene in positions –1082, –819 and –592, leads to a decrease in cytokine production and a predisposition to UC [2, 8, 9, 10, 11].

THE AIM

To investigate of SNPs of TLR-2,3,4, NOD2/CARD15, JAK2, and IL-10 in patients with early and late UC onset.

MATERIALS AND METHODS

126 patients with UC were investigated where during period (6 years). Informed consent was obtained from all patients before the start of the investigation. The most of patients with UC -62 (49.3%) had established diagnosis between 17 and 40 years (A2), and 52 (41.2%) patients had (A3), but only in 12 (9.5%) patients had diagnosis of UC was performed in childhood (Table I).

The location of UC characterized by the inflammation in the large intestine. Proctitis and proctosigmoiditis had 52 (41.3%) patients (E1) from left-sided UC suffered (E2) 32 (25.4%) persons and 42 (33.3%) patients had pancolitis (E3). Patients with moderate severity of UC also predominated among all UC patients – 52 (41.3%)

persons. Mild severity of disease occurred in 40 (31.7%) patients. Only 34 (27.0%) patients had severe UC. Index Mayo in all UC patients group consisted 2.6±0.9 score points. Severity of UC has correlated with extensive character of inflammation in the large intestine. As the majority of UC patients had moderate activity of disease, endoscopical index (EI) was equal to 2.1±0.5 in a whole group of patients with UC.

To assess the predisposition of the early and late onset of UC the incidence of the following SNPs: *Arg753Gln TLR2* gene, *Phe412Leu* TLR3 gene, *Asp299Gly* and *Thr399Ile TLR4* gene , *C-819T, G-1082A* and *C-592A* gene *IL-10*, *Val617Phe* gene JAK2, *Gly908Arg* gene NOD2/CARD15 were analyzed.

To highlight SNPs and used the polymerase chain reaction (PCR) technique DNA was extracted by the method of phenol-chloroform extraction, which was subsequently washed with 70% ethanol solution. After drying in air, further dissolution was carried out in deionized water, storage took place at a temperature of -20 °. Amplification of PCR sequences were carried out by thermocycler (Corbett, Australia), using the Litex (Russia) genotyping kits according to the instructions. To analyze the obtained amplification data, electrophoresis was used in a 2% agarose gel, which was stained with ethidium bromide, and a ultraviolet transilluminator was used for scanning. Characteristics of the studied polymorphic variants of genes were present in table II.

Table I. Clinical characteristics of UC patients

Disease	UC
Total number	126
Male/Female	1.3:1
Age of onset	
Mean (years)	48.3±9.2
Below 16 (A1)	12 (9.5%)
17- 40 (A2)	62 (49.3%)
Above 40 (A3)	52 (41.2%)
Location	
Distal colon (E1)	52 (41.3%)
Left-sided (E2)	32 (25.4%)
Pancolitis (E3)	42 (33.3%)
Severity (index)	Mayo
Total	2.6±0.9
Mild	40 (31.7%)
Moderate	52 (41.3%)
Severe	34 (27.0%)

RESULTS

Out of 126 patient 76 had early onset (before 35 years) and 50 patients had late onset of disease (after 50 years). Most of the patients had TLRs polymorphism. SNPs of TLR3 were observed in 50.8 % cases. TLR 4 (*Thr399Ile*) polymorphism was more common than TLR3, and was observed in 81 (64.3%) UC patients. At the same time, polymorphism of NOD2/CARD15 and IL-10 genes were revealed with almost the same frequency – 49 (38.9%) and 50 (39.9%) patients, respectively (Table III).

In patients with early onset of the disease TLR4 *(Thr399Ile)* and IL-10 polymorphism prevailed – 60 (78,94%) and 34 (44.73%) patients, respectively. In patients with late onset of the disease had significantly often polymorphism of TLR-2, 3 and 4 – 35 (70.0%), 33 (66.0%) and 25 (50.0%) patients, respectively. At the same time, there was a significant difference in the frequency of TLR2 gene polymorphisms in patients with early and late onset of UC (27.63% and 70.0%, respectively ($p<0.05$).

JAK2 and NOD2/CARD15 polymorphism was observed less common than changes in the TLR genes. At the same time, JAK2 polymorphism was more often detected in patients with early onset of UC 24 (31.6%), NOD2/CARD15 polymorphism prevailed in patients with late onset of the disease – 23 (46.0%) patients ($p <0.05$). The polimorphism of the IL-10 gene was more typical for patients with early disease onset 44,7% ($p <0.05$).

Despite the revealed differences in SNPs in different age categories of patients with UC, it should be noted that the average number of polymorphisms in one patient were not significantly different from patients with early to late onset disease, although it was slightly higher in patients with early onset UC (2,76 ± 0,13 ($p<0,1$)).

DISCUSSION

The association of NOD2/CARD15 gene polymorphism was the first with proven association with IBD [2, 12].Initially, the polymorphism of this gene, accompanied the development of Crohn's disease. However, recent studies have shown that polymorphism of the NOD2/CARD15 gene correlates with the development of UC. In our research it was revealed that NOD2/CARD15 SNPs are more common in patients with late onset of the disease that is corresponding with the other references [2, 13].This gene belongs to the family of pattern-recognizing receptors (PRR), which also include TLRs. Mutations in NOD2 presumably increases sensitivity to CD-receptors that can lead to changes in the immune response, the permeability of the intestinal mucosa barrier, causing the severety of disease.

TLRs are involved in the recognition of components of the cell wall of bacteria and viruses, in the activation of a cascade of protective mechanisms of local and systemic immunity [14]. TLRs are widely represented on the surface of the intestinal epithelium, on monocytes, macrophages [9]. The binding of receptors to a bacterial antigen leads to the activation of NF-kB and JAK2. JAK is involved in the phosphorylation of the membrane receptor, facilitating the coupling of STAT-proteins to the homologous domain of the phosphorylated cell membrane receptor. Phosphorylated STAT-proteins dimerize and translocate to the nucleus to regulate gene expression. Thus, the sequential cascade of phosphorylation leads to the activation of transcription factors, alters the expression of pro-inflammatory cytokines, and triggers the mechanism of the

Table II. Characteristics of the polymorphic variants of genes

Gen	Localisation	Polymorphism	Primer structure	Reaction fragment
TLR2	10q24.1-24.3 Ecson 5	Arg753Gln	5′-aat-tac-aac-cag-agc-ttg-gc 5′-tat-cac-ttt-cca-taa-aag-caa-g	SmaI
TLR4	10q24.3-qter 5′-flanking region	Asp299Gly	5′-cca-gtc-gag-tct-aca-ttg-tca 5′-ttc-att-ctg-tct-tct-aac-tgg	PstI
	10q24.3-qter Intron 6	Thr399Ile	5′-ctg-ctg-cta-atg-gtc-act-tg 5′-gga-gtt-caa-gac-cag-cct-ac	DraI
IL-10	C-819T	Deletion	5′-tgc-ttc-acg-tgt-tat-gga-ggt-tc 5′-gtt-ggg-ctc-aaa-tat-acg-gtg-g	-
IL-10	G-1082A	Deletion	5′-ggt-cat-tct-gaa-ggc-caa-gg 5′-ttt-gtg-gac-tgc-tga-gga-cg	-
TLR3	Phe412Leu	313AG	5′- gta-gtt-tgc-cca-agg-tca-ag 5′- agc-cac-ctg-agg-ggt-aag	BsoMAI

Statistical analysis was performed using the standard software package for statistical analysis MedStat.

Table III . SNPs in patients with late and early onset of UC

SNPs	Total, n = 126		Early onset, n = 76		p<	Late onset, n=50		p1<	p2<
	n	%	n	%		n	%		
Tlr2	56	44.4	21	27.6	0.05	35	70.0	0.05	0.05
Tlr3	64	50.8	31	40.7	0.1	33	66.0	0.05	0.5
Tlr4Asp299gly	45	35.7	20	26.3	0.05	25	50.0	0.05	0.05
Tlr4Thr399Ile	81	64.3	60	78.9	0.05	21	42.0	0.05	0.01
IL-10	50	39.9	34	44.7	0.1	16	32.0	0.1	0.0 5
NOD2/CARD15	49	38.9	26	32.9	0.1	23	46.0	0.1	0.05
JAK2	36	28.6	24	31.6	0.1	12	24.0	0.1	0.05
The average per patient	2,43±0,28		2,76 ± 0,13		0,05	2,11 ±0,32		0,05	0,05

p – differences between group of patients with early onset of UC and total group
p1 - differences between group of patients with late onset of UC and total group
p2 - differences between group of patients with early onset of UC and late oncet group

inflammatory response [2, 13]. TLRs activation promotes the synthesis of pro-inflammatory cytokines, including IL. TLR4 is found not only in the intestine, but also on cardiomyocytes, in the brain, on leukocytes in peripheral blood, TLR3 only on dendritic cells as the primary link in contact with the antigen. Thus, the violation of the pattern of the pattern-recognition domain TLR4 and the Asp299Gly Thr399Ile polymorphism are associated with the risk of UC [10]. TLR4 Asp299Gly gene polymorphism changes the resistance of a microorganism to Gram-negative bacteria, thereby contributing to the occurrence of dysbiosis. When single nucleotide substitutions appears in the TLR 2 gene, the susceptibility of the immune system to infectious agents changes. The significant difference between the frequency of gene polymorphisms TLR 4 was revealed our study: in patients with early onset prevailed polymorphism *Thr399Ile* TLR 4 gene, and in patients with late onset – *Asp299Gly* SNPs. At the same time, the difference in TLR3 polymorphism in patients with early and late onset was not significant. It should be noted that patients with late UC onset noted an increase in the frequency of polymorphisms of TLR genes except *Thr399Ile* gene TLR4 which is more character for young UC population [1, 9, 13].

Changes in these genes contribute to the violation of colonization and colonization resistance of the intestinal microbiota, which creates the basis for the formation of intestinal inflammation. On the other hand, the increased frequency of these polymorphisms were revealed in patients with late UC onset, thus, in the second half of life, which were not so common for patients with early UC onset. Consequently, we can assume the epigenetic effects of environmental factors on the gut microbiota formed on the background of long-term intestinal dysbiosis. This might lead to unspecific latent inflammatory process in the lamina propria with insensibly progredient modification of immune response and manifestation of UC in the elderly age.

It should be noted that the polymorphism of the IL-10 gene was more frequently detected in patients with an early onset of the disease, which could determine the relative insufficiency of the anti-inflammatory cytokine response in these patients [11, 15]. Based on the fact that the JAK2 system is involved in the synthesis of nuclear transcription factors and the formation of the immune response, including the expression of proinflammatory cytokines, the combination of JAK2 polymorphism and decreased synthesis of IL-10 can lead to a modified immune response

in the direction of enhancing the proinflammatory component that is observed in patients with early UC onset. In patients with late onset of UC the pattern recognizing receptor polymorphisms not only SNPs of TLRs genes, but also NOD2/CARD15 were dominant. This can change not only the presentation of the antigen, but also modify the formation of inflamasoma with a change in the differentiation of immunocompetent cells, stimulation of the pro-inflammatory component of the immune response in patients with late onset of UC.

CONCLUSIONS

SNPs were detected in patients with early and late onset of UC. The frequency of polymorphisms occurrence of individual genes was different. Polymorphisms of pattern-recognizing signal receptor TLR2, 3 genes and TLR4 Asp299Gly, NOD2/CARD15 prevailed in patients with late UC onset that allow to suppose that bacterial flora might play one of the key role in modification of immune response and contribute to the development of UC. In patients with early UC onset polymorphisms of the JAK2 and IL-10 genes prevailed responsible for the cytokine cascade activation and start the immune mechanism that might lead to a more aggressive course of the disease in such patients.

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This article is carried out within the framework of scientific research work: clinical and pathogenetic aspects of diagnostic and treatment of patients with a comorbidity diseases (cardiovascular, digestive, endocrine system), clinical and pathogenetic aspects.

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ORIGINAL ARTICLE
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AGE AND SEX-RELATED STRUCTURAL AND FUNCTIONAL CHANGES OF BONE REMODELING DURING SIMULTATE ABDOMEN CT-SCANNING

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ABSTRACT

The aim of investigation was to study the structural and functional conditions of cortical and trabecular layers of lumbar vertebrae L_1-L_5 in different age groups.

Materials and methods: In order to assess BMD of the lumbar vertebrae 102 people18 to 75 years old was examined. Study of bone mineral density cortical and trabecular layer of lumbar vertebrae (L_1-L_5) performed by computed tomography in Hounsfield Units (HU) in terms of standard deviation (SD).

Results: The results of computed tomography showed a direct relationship of bone mineral density of lumbar vertebrae with age of examined persons. Osteoporosis and osteopenia was registered in 15% of men and 30% women in middle adulthood, in late adulthood – 35% and 50% respectively. During early old age osteopenia and osteoporosis are observed in 37,5% and 25% of men and 26% and 64% women. Osteoporosis in the middle old age has been reported in 50% of men and 75% of women

Conclusions: Results of the study showed a direct link between mineral density, age and gender. The middle adulthood age period was characterized by the highest mineral mass compared with the other age periods. Then there is a loss of bone mass throughout life, and with the onset of aging osteopenia and osteoporosis are recorded. It was investigated that in the early old age, loss of bone mass is mainly observed in female.

KEY WORDS: bone, bone mineral density, remodeling

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INTRODUCTION

Bone tissue is a metabolic active system and its functioning ensured by the process of remodeling, which consists in the continuous replacement of bone plates, the formation of new osteons and trabeculae on the site of the resorbed ones. This process maintains a constant mineral bone mass throughout the entire life [1, 2, 3]. The structural and functional conditions of bone tissue depends on many factors, epidemiological studies indicate the dependence of the formation of bone tissue on age, sexual, ethnic characteristics, etc. [4, 5]. Randomized, multicentre studies in Europe and the United States have demonstrated that in the puberty and post-puberty period, bone mass is actively increasing, reaching its maximum by an average of 25-30 years. Then comes the period of equilibrium. The physiological decline in bone mass begins at approximately 35 years of age and increases in the first 5-10 years after menopause in women, 2-3% per year. In men loss of bone tissue is 0,3-0,4 % per year. From 65-70 years, the rate of loss of bone tissue is reduced and make 0,2-0,3 % per year for both sexes [6, 7, 8].

The imbalance of bone remodeling leads to the formation of osteopenia and osteoporosis – systemic or local disease of the skeleton with decreasing of bone mineral mass. This leads to increasing of bone fragility with subsequent risk of fractures. Particularly in women, the development of osteoporosis is associated with loss of bone mineral density (BMD), which occurs most often during menopause. At the same time, the deficiency of estrogen leads to a slower inhibition of osteoclasts, a decrease in the activity of osteoblasts, increases the sensitivity of cells to parathyroid hormone, enhances the synthesis of pro-reactive cytokines (IL-1, IL-6, IL-11, TNF) [9, 10].

Recent research shows that calcium, iodine, zinc and fluoride deficiencies, as well as sedentary lifestyle and bad habits can also affect the bone mineralization even in childhood and adolescence. In particular, the effect of iodine deficiency on bone tissue is indirect, but it plays a key role in the functioning of the thyroid gland hormones that are necessary in the regulation of normal growth for the development of the skeleton [11]. The problem of hypothyroidism is relevant for the endemic regions of Ukraine, which includes the Lviv region, where iodine deficiency tends to increase. Therefore, this problem is relevant for further our scientific work.

THE AIM

Investigate the structural and functional conditions of the cortical and trabecular layers of the lumbar vertebrae (L_1-L_5) in persons of different age groups living in the Lviv region.

MATERIALS AND METHODS

In order to assess the BMD of the lumbar vertebra, 102 patients aged 18 to 75 years were examined. The patients

Fig. 1. Vertebral attenuation measurement of BMD (HU) using the ROI method

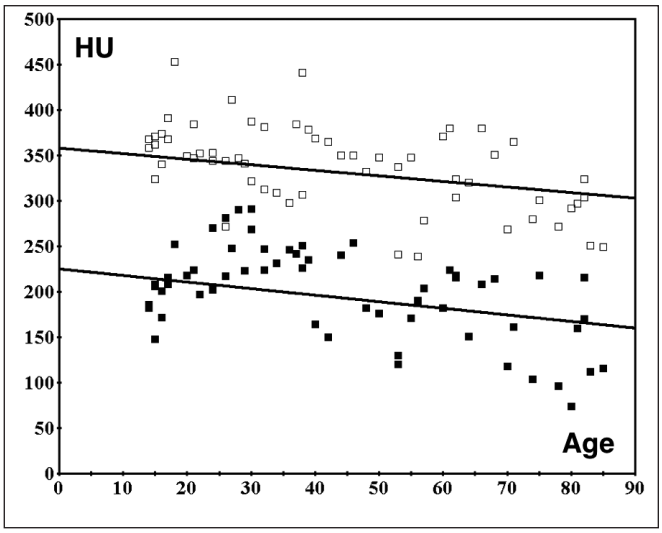

Fig. 2. BMD (HU) of trabecular (■) and cortical (□) layers L1-L5 in men

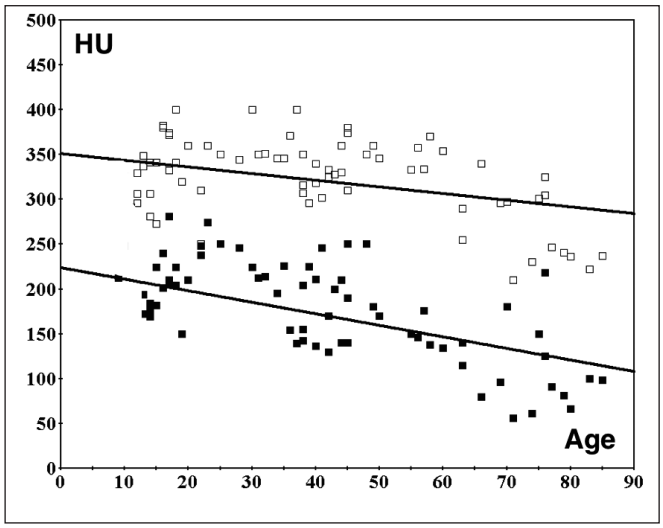

Fig. 3. BMD (HU) of trabecular (■) and cortical (□) layers L1-L5 in women

were divided into 10 groups that consisted of 5 age periods: early adulthood (18-30 years), middle adulthood (30-45 years), late adulthood (45-60 years), early old age (60-75 years) middle old age (75-90 years). Investigation of mineral density of lumbar vertebrae bone tissue (L_1-L_5) was performed by computer tomography (CT) GE / e Dual CT scanner using a standard gauge with a thickness of 3 mm, a peak voltage of 120.0 kV and a current of 496 mA. Data was processed using the eFilm Workstation 3.4.0, 34 bit (Merge Healthcare, USA, 2013) program on the Windows 7 Professional SP 1, 32 bit (Microsoft, USA, 2009) system.

The instrument was calibrated using phantoms of water, air, ethanol and potassium phosphate, which were scanned together with the subject. The values of the sections were corrected by the formula:

$$\mathbf{HU = 1000\ (HU_b - Hu_w)\ /\ HU_w - HU_a}$$

where:

HU_b – the average density of the scanning area of the spongy bone,

HU_w – the average density of the standard water in the phantom,

HU_a – the average density of the standard air in the phantom.

The result of each subject was calculated by the formula:

$$\mathbf{HU = P_1 HU_1 + P_2 HU_2 + \ldots + P_n HU_n\ /\ P_1 + P_2 + \ldots + P_n}$$

where:

P – the number of pixels in the area of the standard scan.

Density indices of the bone tissue of the trabecular layer of the lumbar vertebrae were determined according to CT criteria in the units of Hounsfield (HU) – ROI method, with standard deviation (SD), fig 1.

Table 1. Bone mineral density of the lumbar vertebra, HU

№	Age periods	BMD (HU)			
		Men		Women	
		Trabecular bone	Cortical bone	Trabecular bone	Cortical bone
1.	Early adulthood	223,0 ± 8,4	381,6 ± 21,8	203,9 ± 9,6	367,9 ± 10,0
2.	Middle adulthood	245,0 ± 9,9	367,4 ± 13,3	232,8 ± 7,7	360,1 ± 12,8
3.	Late adulthood	197,8 ± 16,5	331,5 ± 13,7	180,9 ± 8,9	341,2 ± 6,2
4.	Early old age	179,3 ± 15,5	328,1 ± 13,7	119,6 ± 13,2	303,1 ± 17,2
5.	Middle old age	145,3 ± 20,5	287,0 ± 10,3	116,0 ± 18,3	262,8 ± 14,2

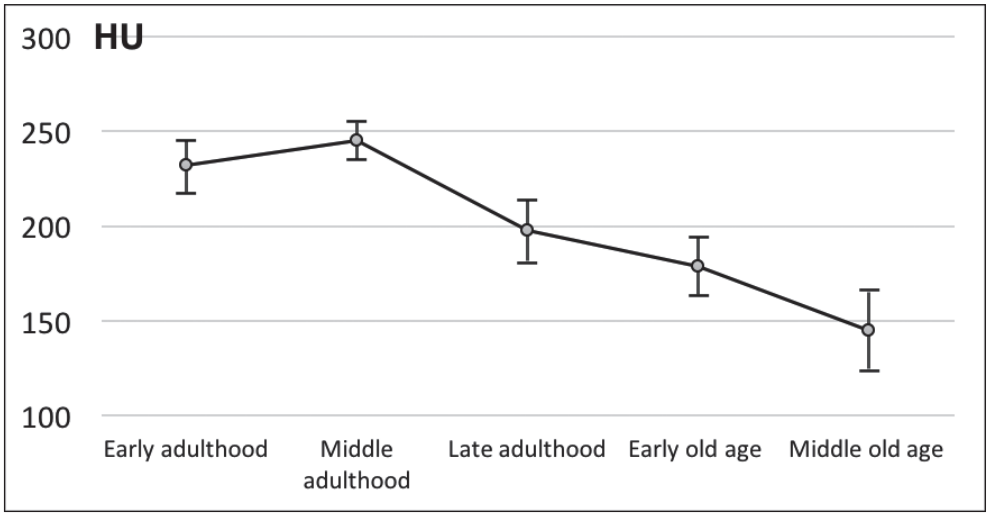

Fig. 4. Age dependence of BMD (HU) of lumbar vertebrae in men

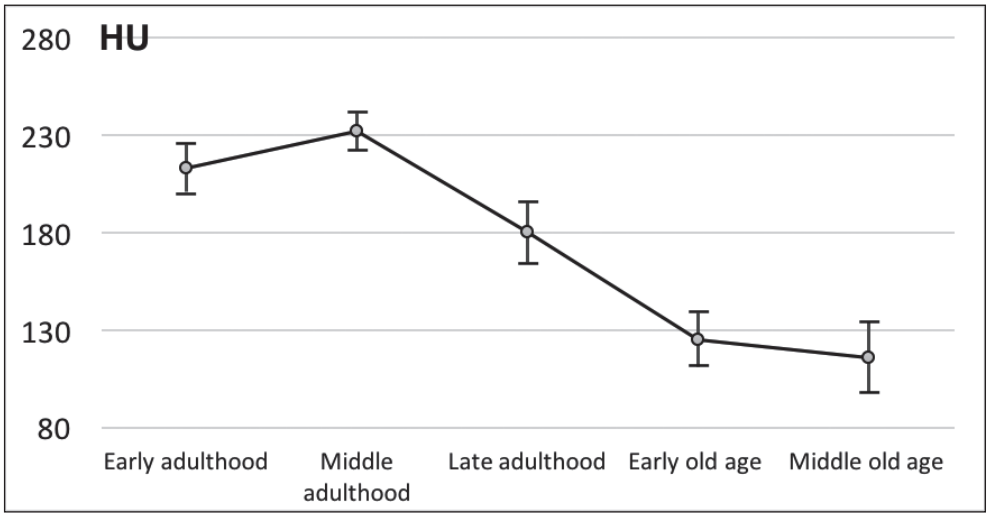

Fig. 5. Age dependence of BMD (HU) of lumbar vertebrae in woman

Average values of the mineral density were more than + 216.7 – considered as normal BMD (> -1 SD), the value from + 216.7 to +148.6 – osteopenia, (-1 – (- 2,5) SD) and were less than + 148.6 – osteoporosis (≤ 2.5 SD) [1, 10].

RESULTS AND DISCUSSION

The results showed a direct dependence of BMD of the trabecular and cortical layers of the lumbar vertebrae to the age and sex. It has been established that mineral bone mass is actively increasing in early adulthood, tab. 1, fig 2, 3.

In males of this age group, the mineral density of bone tissue was 223,0 ± 8,4 for the trabecular layer and 381,6 ± 21,8 for the cortical layer, respectively. For females: for the trabecular layer – 203,9 ± 9,6 and for the cortical layer 367,9 ± 10,0, respectively. Middle adulthood was characterized by the highest mineral mass, compared with other age periods. Mineral density – 245,0 ± 9,9 and 367,4 ± 13,3 for trabecular and cortical layers in males and 232,8 ± 7,7 and 360,1 ± 12,8 females, respectively. As a result, osteopenia was recorded in 15 % of the examined men and in 30 % of the female population.

Late adulthood is characterized by a gradual loss of bone mass. Especially this dependence is observed in the examined subjects of the female sex. In particular, osteopenia was detected in 35% of men and 52% of women, and osteoporosis – 12% and 24% respectively. The reason

for accelerated bone remodeling in this age is the lack of sex hormones, which is typical during menopause. The average density of bone tissue in the subjects of the late adulthood was 197,8 ± 16,5 and 331,5 ± 13,7 for men and 180,9 ± 8,9 and 341,2 ± 6,2 for women. During early old age osteopenia and osteoporosis are observed in 37,5% and 25% of men and 26% and 64% of women. Osteoporosis in the middle old age has been reported in 50% of men and 75% of women, fig 4, 5.

CONCLUSIONS

The mineral density of the trabecular and cortical vertebrate layers in male and female was estimated using CT. It has been established that mineral bone mass is actively increasing in early adulthood. The middle adulthood age period was characterized by the highest mineral mass compared with the other age periods. Then there is a loss of bone mass throughout life, and with the onset of aging osteopenia and osteoporosis are recorded. It was investigated that in the early old age, loss of bone mass is mainly observed in female subjects. The reason for accelerated bone remodeling in this age is the lack of sex hormones that occurs during the menopause.

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ORIGINAL ARTICLE
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PLACENTAL DYSFUNCTION: HEALTH STATUS, NUTRITIONAL STATUS AND MINERAL PROFILE OF A MOTHER-CHILD PAIR

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ABSTRACT

The aim: the analysis of the PD, pregnancy, the labor, the research on peculiarities of the development and health status of breast-fed children, who are born to mothers with PD, by means of analyzing the mother-child pair's nutritional status and mineral homeostasis.

Materials and methods: At the 1 stage, an analysis of the PD frequency, the pregnancy, the labor was conducted during 5 years. At the 2 stage, 188 mother-child pairs were examined: 84.04% women had PD and 15.96% didn't have it. The research included the analysis of the anamnestic data, maternal nutritional status, general clinical study, assessment of the physical, psychomotor level of the child's development, study of the elemental profile.

Results: High frequency of complications in pregnancy and labor was observed in cases when women had PD, due to the imbalance in the "mother-placenta-fetus" system. The results' analysis showed an increased level of Zn (1.437%), K (10.147%), and Ca (83.900%) in hair; an increased level of K (82.818%), Cr (0.274%), and Na (3.611%) in breast milk of women with PD. Children born to mothers with PD had a significantly increased level of Cr (0.92%), S (0.578%) and P (0.169%), Na (0.107%), Ca (56.041%), and Zn (7.149%).

Conclusions: PD has a negative impact on the pregnancy and labor and may be one of the factors causing the mineral imbalance of breast-fed infant.

KEY WORDS: mother-child pair, placental dysfunction, state of health, nutritional status, mineral profile

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INTRODUCTION

At present, the issue of studying the medical and social risk factors in terms of developing interdependent disorders in a mother-child pair's state of health is raised in separate works, which only fragmentarily show the essence of the problem concerning the effects of pregnancy complications on the child's health. The most pressing issue of modern obstetrics is the placental dysfunction (PD), since it is the main cause of complications during the pregnancy and labor, it greatly increases the incidence of perinatal morbidity and mortality, leads to complications in the postnatal period, fosters disability cases of a newborn child. PD can promote fetal programming to develop adverse health consequences in childhood [1,2]. According to recent studies, during the pregnancy external factors, including microbial agents, significantly affect the fetal-placental complex and cause the development of newborns' health complications that allows the placenta to be considered as the primary route of prenatal transmission of damaging factors from mother to fetus [3]. If the fetus survives, the child may have an increased risk of morbidity throughout the life [4]. At present, the effect of a mother's nutrition and her past diseases on the placenta development has been proved [5]. That is why ensuring the adequate nutritional status of women during pregnancy is one of the essential and obligatory conditions for the normal placenta functioning and, accordingly, the birth of a healthy child.

Thus, the study of the health of women, as well as of children born to mothers with placental dysfunction and PD impact on the further development of the child, is an urgent issue that caused the conduct of this research.

THE AIM

The aim is to conduct a retrospective analysis of the PD cases, pregnancy course, the labor and the state of newborns, the research on peculiarities of the development and health status of children that are born to mothers with placental dysfunction and are exclusively breast-fed, by means of analyzing the mother-child pair's nutritional status and mineral homeostasis

MATERIALS AND METHODS

At the first stage, a retrospective analysis of the PD frequency, the course of pregnancy, the labor and the state of newborns during 5 years (examining 14276 women) was conducted to analyze the prevalence of this pathology.

To clarify the mechanisms of PD development and the development of clinical and pathogenetic approaches to

its prevention, 188 "mother-child" pairs were examined. The experimental group consisted of 158 (84.04%) mother-child pairs with mothers having the PD; the control group – 30 (15.96%) pairs with mothers having uncomplicated pregnancy.

The general clinical study of pregnant women was conducted in accordance with the Order of the Ministry of Health of Ukraine No. 503 of 28.12.2002 [6].

All pregnant women, in addition to the general clinical and laboratory examination, underwent dynamic ultrasound and doplerometric examinations, a cardiotocography (CTG), and examinations to determine the biophysical profile of the fetus (BPP). To interpret cardiotocograms, a point rating system by W. Fisher (1976), that was later improved by I. O. Makarov, was used [7]. Prenatal screening was conducted to determine the possible risk of birth of a child with congenital malformations; screening included the identification of biochemical, ultrasound markers of chromosomal pathology during 11-13 weeks, geneticist's counseling at 20th-22nd week. In addition, the identification of women's mineral profile during pregnancy and lactation was conducted by determining the relatively toxic (Al, Ti, Ba, Pb, Sr) and essential (Fe, I, C, Zn, Co, Cr, Mo, Ni, V , Se, Mn, As, F, Si, Br and Li) elements in samples of hair and breast milk by spectrometry method using the ElvaxLight apparatus (Ukraine, 2008). Hair was chosen as a biological material for research, since it reflects the long exposure of the elements in a human body.

The second stage of the research involved examining a "mother-child" pair when the child was 5 months of age, namely, before the introduction of complementary feeding, and it included, in addition to analysis of anamnestic data, general clinical examination with an assessment of the level of physical and psychomotor development of the child, ME study: for children – using the hair; for mothers – using the hair and breast milk to determine the mineral profile of a "mother – child" pair.

Statistical processing of the obtained results was carried out by methods of mathematical statistics. For each statistic series, the arithmetic mean (M) was calculated as well as the mean error of the arithmetic mean (m). The estimation of the probability of differences in average mean in groups (p) was calculated using Student's criterion (t). The difference was considered probable if a value of $p < 0.05$. Statistical processing of the obtained research results was performed on a personal computer using Microsoft Excel program.

RESULTS AND DISCUSSION

According to a retrospective analysis, over the past 5 years the frequency of PD among pregnant women has increased from 5.91% to 15.35%. In order to determine the possible influence of PD on the incidence rate among newborns, it is necessary to understand the structure of morbidity of mature and premature infants. According to the results of the retrospective analysis based on the work of the city's perinatal center, over the past 5 years the number of the fetal growth restriction syndrome (FGR), caused by PD in infant newborns (14.8%), has increased significantly compared with its number 5 years ago (5.8%). The incidence of congenital pneumonia increased from 1.2% to 3.4%, the incidence of intrauterine infection – from 1.7% to 2.02%. Among premature infants the incidence of intrauterine infection increased from 10.4% to 15.7%, FGR frequency increased from 7.3% to 12.5%. The main causes of intrauterine fetal death were: chronic fetoplacental insufficiency caused by infectious lesions, preeclampsia, extragenital pathology; congenital malformations of the fetus, umbilical-placental circulation disorders. The placental dysfunction has led to FGR and subsequent fetal death in 30.3% of cases. Conducting histological examination of placenta, markers of placental dysfunction were detected in 50.2% of cases, infectious lesions (exudative villusitis, pyogenic chorioamnionitis) were detected in 66.7% of cases. It is morphologically proved that PD leads to perivascular hemorrhages, angiomatosis, pyogenic necrotic deciduitis, funiculitis, or is a consequence of perinatal infections [8].

The results analysis of the examination of the "mother-child" pairs showed that the pregnant women in the experimental group were from16 to 42 years of age that amounted to an average of 28.4 ± 5.2 years old. In the control group, the pregnant were 22.6 ± 4.5 years old ranging from 18 to 36 years old. A significant proportion of pregnant women, who are over 30, draws the attention: in the experimental group -39.2% of women, whereas in the control group – 16.7%. The ratio of resident of the city and the country in the experimental and the control groups was unlikely to differ ($p > 0.05$). The largest part in both groups was represented by housewives, which may be due to socio-economic conditions that have developed recently in Ukraine. Living conditions of the examined women were satisfactory, 16 women of the experimental (10.1%) group smoked before the pregnancy, 2 (1.3%) – during the pregnancy. Alcoholic beverages were not abused. Anemia of varying severity was observed in 23 (14.6%) women in the experimental group, while in the control group, it was observed in 2 (6.6%) women, which could be considered as one of the causes of PD in pregnancy.

Among women of the experimental and the control groups, the primiparous ones were 79 (45.6%) and 12 (40%) women, which indicates the comparability of the given groups. Analyzing the previous pregnancy courses of the primiparas, it was determined that the previous pregnancies of women in the experimental group ended mostly in spontaneous or artificial abortions, missed miscarriages.

During doplerometric examination of uterine arteries, an increase in vascular resistance was observed in 70.9% of pregnant women with PD. Quantitative evaluation of blood flow in the umbilical artery showed that the amount of the pulsation index during the second and third trimesters varies from 1.8 to 2.10. Similar data were found in dopplerometric results of blood flow of the mesencephalic artery and fetal aorta. Thus, the dopplerometric study clearly demonstrates the negative changes in the blood flow in the mother-placenta-fetus system of pregnant women with PD.

On measuring the BPP, attention was drawn to non-

stress tests, breathing movements, fetal motor activity, tonus, amniotic index and placental maturity degree. The last two indicators characterize the degree of the PD. Rate of the BPP using the scale by A. Vintzileos (1987) ranged from 8.8 ± 0.2 points in the experimental group to 11.9 ± 0.1 points in the control group ($p < 0.05$).

Comparative analysis of fetal CTG of pregnant women with PD showed severe disorders of the fetus in 11.4% of cases and moderate ones in 26.6%. It should be noted that 142 (89.9%) women in the experimental group had infectious inflammatory diseases of the reproductive system, diagnosis of which was carried out on the basis of complaints, clinical picture, laboratory test results.

Among the pathogens staphylococcus, streptococcus, enterococcus, coli-bacillary infection, herpes causative agents, gardnerellosis, toxoplasmosis prevailed. The analysis of pregnancy course of women with PD showed the following complications: vomiting of pregnant women – 36.7%, threatened miscarriage – 30.4%, the threat of preterm labor – 45.6%, preeclampsia – 22.8%, fetal distress – 18.4%. Labor analysis showed that preterm labor occurred in 13.9% of the pregnant women in the experimental group, fetal distress – 51.3%, premature rupture of fetal membranes – 9.5%. Methods of labour: in 84.2% of cases – vaginal birth, 15.8% – cesarean section. The fetal state was assessed to 8 points and above according to the Apgar score in 70.9% of cases. In other words, the analysis of pregnancy course and labor among women with PD showed a high incidence of complications of pregnancy and labor, which is caused by the imbalance in the mother-placenta-fetus system, which coincides with the data of other researchers [9].

The results of the maternal nutrition questionnaire using the Dietplan-6 program (https://dietplan6.software.informer.com/6.6/) showed that 60.1% of women with PD had a lack of calcium, iron, magnesium and protein in their diet , and 30,2% – lack of vitamins B6, B12, E, A and D, 58,6% – iodine deficiency. Among mothers who did not have a PD in their medical history, an imbalance of the diet was observed much less frequently: 20.1% of women had reduced calcium, magnesium, iron consumption; 5.6% had a lack of protein, vitamin C, fats; 15.2% – of iodine, vitamins D, B6 and B12.

The result analysis of the mineral profile research of mothers with PD showed ME imbalance due to an increase in levels of Zn (1.437%, $p \leq 0.005$), K (10.147%, $p \leq 0.005$), and a decrease in Ca (83.900%, $p \leq 0.005$) level, whereas the mothers who did not have PD, had the level of Zn / K / Ca within the normal range (0.6667% / 9.133% / 84.913%, $p \leq 0.005$).

A noteworthy aspect was that in hair samples of mothers with PD there was a decrease in levels of Cr (0.083%, $p \leq 0.005$) and Na (1.820%, $p \leq 0.005$), in contrast to hair samples of mothers without PD, where only Cr level decrease is observed (0.114%, $p \leq 0.005$). According to the results of breast milk spectrometry, it was determined that samples of the mothers with PD showed increased levels of K in 82.818% of cases, Cr – 0.274%, Na – 3.611% (respectively, $p \leq 0.005$), and samples of the mothers without PD showed an increase in levels of K in 80.631% of cases, Cr – 0.251%, Na – 2.529% (respectively, $p \leq 0.005$).

It should be noted that, the allegedly toxic ME: Ti (0,043%, $p \leq 0,005$), Sr (0,068%, $p \leq 0,005$) and toxic Pb (0,002%, $p \leq 0,005$) were detected in the hair of mothers of both groups. Ti (0.024%, $p \leq 0.005$) and Pb (0.006%, $p \leq 0.005$) were detected in the breast milk of the mothers of both groups,.

Anamnesis analysis of children born to mothers with PD showed that 16.4% of children had the manifestation of atopic dermatitis, 9.1% had several cases of acute obstructive bronchitis, 5.3% had laryngotracheitis, 15.5% had rhinopharyngitis, 8.0% – hip dysplasia. In a group of children born to women without PD, 4.2% of children had manifestation of atopic dermatitis and 1,3% – hip dysplasia.

Assessing the level of physical development of the children, it was found that 3.7% of the children whose mothers had a medical history of PD, had a debilitation. 12.76% of children born to the mothers, who did not have a PD during the pregnancy, were diagnosed to be prone to possible overweight. Neuropsychic development of children of both groups was up to the age. As to anamnestic data, 8.3% of children born to mothers with PD were known to have hypoxic-ischemic central nervous system (CNS) injury.

The results analysis of hair spectrometry showed that children born to mothers with and without PD didn't have significant differences in the levels of Mg, S, Cl, Fe, Cu, Ni, Sr, Pb, Br, Ci, Si and Mo.

At the same time, it was determined that the children born to mothers with PD had the increased content of Cr – 0.928% ($p \leq 0.005$), S-0.578% ($p \leq 0.005$) and P – 0.169% ($p \leq 0.005$) in comparison to the same indicators of the children born to mothers without PD: S – 0.462% ($p \leq 0.005$), Cr – 0.381% ($p \leq 0.005$), P – 0.016% ($p \leq 0.005$). It was also determined that among children born to mothers with PD there was an increase in levels of Na – 0.107% ($p \leq 0.005$), Ca – 56.041% ($p \leq 0.005$) and Zn – 7.142% ($p \leq 0.005$), which may be connected both with their alimentary intake and the influence of the environment [10,11].

Among children born to women who did not have PD, there were no significant variations in ME values: Na (0.040%), Ca (51.543%), Zn (4.348%), ($p \leq 0.005$).

CONCLUSIONS

1. Placental dysfunction, caused predominantly by intrauterine infectious lesions of placenta, has a negative impact on the course of pregnancy and labor, the state of the fetus, newborn and infant.
2. Mother's placental dysfunction can be one of the factors of imbalance in mineral homeostasis of children aged up to 5 months, which are exclusively breast-fed.
3. The mineral profile of children, who are exclusively breast-fed, depends to a large extent on the nutritional pattern of the mother's diet, both during pregnancy and lactation.
4. Imbalance in mineral homeostasis of children born to mothers with PD, namely, the increase in levels of Zn,

Na, Cr, S, P and Ca, may cause frequent respiratory diseases, a liability to allergic reactions and a disorder of the physical development level in the future.

5. The balance of women's nutrition during pregnancy and lactation should be considered as a means of preventing the mineral imbalance in children.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

PREGNANCY, COMPLICATED BY PREECLAMPSIA: FETOPLACENTAL COMPLEX IMMUNE DEADAPTATION AND HISTOSTRUCTURAL FEATURES

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ABSTRACT

The aim: to study and compare the features of the interleukins levels and morphological changes of placenta at various stages of preeclampsia.

Materials and methods: 109 pregnant women with preeclampsia of varying severity (study group) and 30 pregnant women with uncomplicated pregnancy (control group) were examined. Immunohistochemical method, proinflammatory interleukins levels, morphological and morphometric analysis of peripheral and central placental areas biopsies on the optical and electron-microscopic level have been used.

Results: Morphofunctional changes in the placenta in case of preeclampsia and the increase in the expression level of the transforming growth factor have a series of regular stages from the formation, strain and disruption of adaptive mechanisms with more pronounced signs of morphological immaturity of parenchymal and stromal elements of the placenta, especially in the area of syncytiotrophoblast and spiral vessels. The degree of clinical manifestation of preeclampsia has a correlation relationship with IL-10 deficiency and with the increase in TNF-α, stimulation of macrophage-protein production that contributes to the change in the ratio of Thl / Th2, which are antagonists and inhibit each other's development.

Conclusions: The severity of the preeclampsia course correlates with the state of morphofunctional changes in the placenta and changes in the ratio of the pro- and anti-inflammatory interleukins.

KEY WORDS: interleukins, ultramorphological placental changes, preeclampsia

Wiad Lek. 2020;73(1):99-103

INTRODUCTION

The problem of preeclampsia in pregnant women remains one of the most complex and relevant in obstetric practice [1-6] and requires further study.

Despite constant attention to the problem of gestosis in pregnant women around the world, until now the question of the etiology and mechanisms of the development of pathological processes remain controversial, and none of the existing theories can not convincingly substantiate the causes of this complication of pregnancy. In recent years, more importance in the development of gestosis is caused by a violation of the immune system of the pregnant woman [2].

The urgency of the role of the placenta in the pathological course of pregnancy is extremely high, especially in the development of various gestational complications [3,4,6], such as preeclampsia.

The pre-eclampsia frequency remains high, and this is why the systematic and complex study of morphofunctional placental changes and the state of interleukins in the development of small asymptomatic and atypical pre-eclamptic forms in obstetric practice [4] as factors that can significantly affect the growth of this complication can not be overestimated.

THE AIM

To study and compare the features of the interleukins levels and morphological changes of placenta at various stages of preeclampsia.

MATERIALS AND METHODS

109 pregnant women with preeclampsia of varying severity (study group) and 30 pregnant women with uncomplicated pregnancy (control group) were examined. Used clinical, laboratory, ultrasound, doplerometric, immunohistochemical, biophysical methods of investigation. Morphological and morphometric analysis was carried out in 93 biopsies of peripheral and central placental areas on the optical and electron-microscopic level. Also the state of proinflammatory interleukins (IL)-2, TNF-α and anti-inflammatory – IL-10 in 32 pregnant women with varying degrees of severity were studied. The placenta was taken immediately after delivery and was fixed in Buen's fluid. Paraffin sections were stained with Hematoxylin by Mallory. The quantitative composition of interleukins was estimated by a solid-phase enzyme-linked immunosorbent method. The ELISA test system, DIACLON (France) was used for measurement. The research was carried out according to the manufacturer's methods.

RESULTS

In the study, it was discovered that the usual triangulation of Tsangmejistra was observed in 13% of cases with mild preeclampsia, 28.6% – with moderate and 62.8% of cases – with severe preeclampsia. Combining symptom at all stages of preeclampsia was to some extent hypertension, which varied from 56% of cases with a mild degree of severity up to 98% with severe gestosis. In women with preeclampsia, the increase in the level of proinflammatory interleukins has been noted.

Thus, it was found that proinflammatory IL -2 in the first half of pregnancy in women who make up the risk group for the development of gestosis are from 19.01 ± 1.03 pg / ml (and their level continues to increase in the second half of pregnancy) to 76.36 ± 3.77 pg / ml ($p < 0.05$), and there is a fairly high level of TNF-α throughout pregnancy in women with gestosis compared to the physiological course of pregnancy. In the physiological course of pregnancy, this indicator was lower and was 54.34 ± 3.32 pg / ml. In the development of preeclampsia, it tended to grow from 64.83 ± 3.32 pg / ml at mild progression to 128.81 ± 14.64 pg / ml in severe cases.

At the same time, there is a decrease in the activity of anti-inflammatory interleukins during pregnancy. Thus, IL-10 with 23.66 ± 0.93 pg / ml, which was observed during mild pregnancy, decreased to 18.15 ± 0.92 pg / ml in severe preeclampsia during the second half of pregnancy ($p < 0, 05$), which indicates the corresponding reaction of the main immune mechanisms of the systemic inflammatory reaction.

When conducting postpartum placenta scopia, we note that the decidual septum between the lobes is thickened, the blood vessels of the surface of the mucous membrane varicose changed, there are about 30% hypertrophied villi with destructive changes in the trophoblastic epithelium. Most of the vessels of microvilli are sliced with erythrocytes, they have signs of intracellular edema of endothelial cells and ultrastructural lesions.

Thus, the revealed morphological changes of the placenta indicate an unfavorable course and restriction of compensatory possibilities of the placenta in preeclampsia, and are not signs of intracellular regeneration.

The state of intervertebral gaps is characterized by significant expansion combined with a thickened basal membrane of the cytotrophoblast, expansion of the capillary mesh and vascular edema, expansion of the endoplasmic mesh tubules, and increased content of free ribosomes.

To capillaries with dystrophically altered endothelial cells, activated macrophages adjoined closely. Along with the defeat of the parenchymal and stromal elements of the blood capillaries, changes in the cells of syncytiotrofoblast were manifested. The changes found in the syncytiotrophoblast epithelium showed a development of dystrophic and necrotic changes. The feature of the defeat is the statement of the phenomena of apoptosis. In syncytiotrophoblast, dystrophic changes were observed in the cytoplasm, nucleus and cytoplasmic membrane. Due to pronounced edema in intensticium space, syncytiotrophoblast does not adhere tightly to the basement membrane. On the surface of the cytoplasmic membrane there was a decrease in the number of microvilli. The cytoplasm was characterized by the appearance of vacuoles, which merged with each other and formed the balloon structures (see Figure 1).

In the cytoplasm of the cells there was an uneven location of the organelles. Mitochondria formed so-called mitochondrial complexes, aimed at compensating for losses of energy resources of cells. There were enlarged mitochondria. Cell nuclei had an elongated or rounded shape. Nucleolome was a denser, zigzag form. Nuclear chromatin was located in the form of a depth with a tendency for perinuclear localization (see Fig. 2).

Along with this there were cells with pronounced apoptotic changes. In such cells, the contours of the nucleus become irregular due to the deep invagination of the nuclear membrane. The latter was thinned and hyperosmophilic. The aggregated nuclear chromatin, in the form of large lumps, was located along the inner surface of the nucleolums. Cytoplasm of the cells was hyperosmophilic, condensed.

Endothelial cells looked like thinned cells with a homogeneous cytoplasm. The cytoplasmic membrane is swollen, without clear contours, focal lobed. Often swelling or dehydration of the cytoplasmic matrix was observed with variations in its electron density. The shape and volume of the cell, as well as the desquamation of the fragmented cytoplasm in the lumen of the vessel, were altered. At the same time the number of lysosomes increased in the cytoplasm, indicating the possibility of developing cell autolysis (see Figure 3).

Activated macrophages adjoined closely to capillaries with dystrophically altered endothelial cells. Along with the defeat of the parenchyma-stromal elements of the blood capillaries, changes in the cells of syncytiotrofoblast were revealed.

Thus, the study showed that in women with preeclampsia of moderate severity, there is not only a violation of the rheological properties of the blood and damage to the parenchymal and stromal elements of the vessels of the microcirculatory bed, but also the defeat of the structural elements of syncytiotrophoblast. These changes indicate the tendency of local activation of regenerative processes in the direction of substitution of normal placental tissue as a result of an ischemic injury. In pregnant women with preeclampsia of mild degree, 39.2% to 72.6% of destructively altered villi are found, and at a severe degree their number is even more increasing. In the ultrastructural study, karyopicnosis, caryoreksis of cytoplasm, symplastotrophoblast and basement membrane of trophoblastic epithelium were detected. In the stroma of the villi (according to the stages of preeclampsia), the number of lymphocytes and macrophage-like cells increases progressively, and the number of elements of the fibroblastic series decreases. The described structural changes tended to increase depending on the degree of preeclampsia.

The structural reorganization of the chorionic villi with preeclampsia of mild degree is more often detected

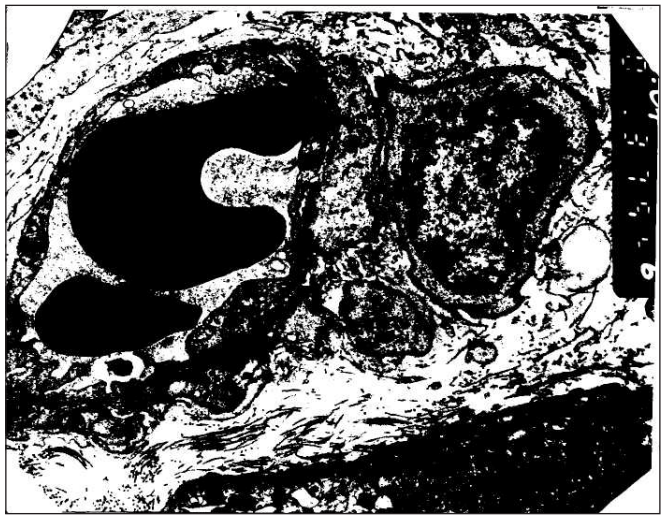

Fig. 1. Capillary with adhesion of erythrocyte to the surface of the endothelial cell. Activated macrophage, balloon structures in swollen interstitial villi in patients with preeclampsia of moderate severity. Electrogram X-12000.

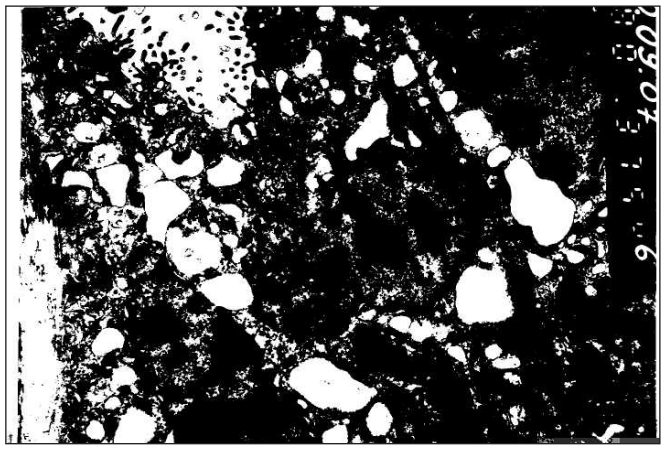

Fig. 2. Syncytiotrophoblast cells with vacuolated cytoplasm, nucleus with chromatin in the form of a lumps in a patient with preeclampsia of moderate severity. Electrogram X-15000.

Fig. 3. Dystrophically altered endothelial cell with numerical lysosomes, edema and felted cytoplasmic membrane in a patient with preeclampsia of moderate severity. Electrogram X-20000.

on the periphery, which allows the central part of the placenta to compensate for these disorders at this stage. In marked hemodynamic violations of hypercoagulation changes in placenta are of a total character with signs of decompensation of its function. Correlation with the use of biophysical methods of research with their comparisons with the histological structure of the placenta suggests that, regardless of the initial mechanism of triggering the pathological process, improvement of the utero-placental blood flow primarily depends on the rheological properties of the blood and the condition of the chorionic villi. The findings are clearly correlated with the state of interleukins, which stimulate a powerful humoral immune response.

Unlike preeclampsia of moderate severity, in preeclampsia of severe degree signs of apoptosis have been observed in a significant number of epithelial cells in the form of sshrinkage of cell membranes, cellular contraction, lysosomal and nuclear fragmentation. Appeared significant amount of cytoplasmic outgrowths and apoptotic cells.. Significant vacuation of the cytoplasm and organelles was observed. The same changes were detected in the syncytiotrophoblast structures. Against the backdrop of structural changes, there was a violation of the rheological properties of the blood with the subsequent development of the "sludge phenomenon", the stasis and the microthrombotic formation, and, in the vessels of the microcirculatory bed, there were small centers of re-endothelialization, and the newly formed cells in the regions of re-endothelialization were characterized by moderate osmophilia, the presence of cytoplasmic processes, increased number of mitochondria, and cluster of lysosomes. The nucleus contained an electron-dense nucleoplasm, a grainy chromatin, aggregated into the lumps. It is located eccentrically in the inner layer of the nuclear membrane. The edges of the nucleus were uneven, perinuclear space was expanded in places. The nucleus had an electron-dense appearance, a compact or loose structure.

The cell membrane of the endothelial cells had microvilli and faint invaginations. The endoplasmic reticulum was represented by short, small tubules. These plate complex contained both small vesicles and extended cisterns, located in different sections of the cytoplasm.

Thus, the study showed that in the fetoplacental complex of women with preeclampsia of severe degree there is a deep defeat of structural elements like blood vessels of hemo-microcirculation with a violation of the rheological properties of blood, and syncytiotrofoblast. Common to them was the same alternative process in the form of apoptosis. However, the beginning of the apoptotic process in the conducted studies had some differences, depending on the structure of the fetoplacental complex. In syncytiotrophoblast, in areas along with nuclei that had signs of apoptosis, there was a disappearance of microvilli, a cytoplasmic seal and the appearance of osmophilic membrane structures. Endothelial cells, the nuclei of which had signs of initial apoptosis, lost intercellular contacts and separated from unaffected cells. Subsequently, the apoptotic process acquired the same characteristics for all cell populations.

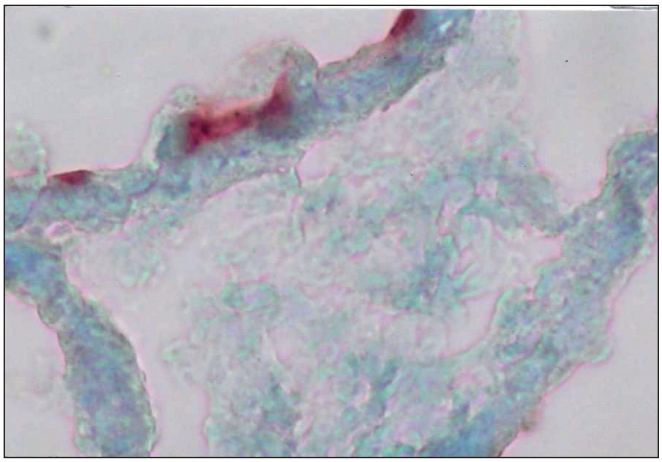

Fig. 4. A fragment of chorionic villus with focal expression of TNF-α antigen in syncytiotrophoblast epithelium (1 – 2 points) in a patient with light preeclampsia. Indirect streptavidin-peroxidase method for detecting TNF-α antigen expression. X – 400.

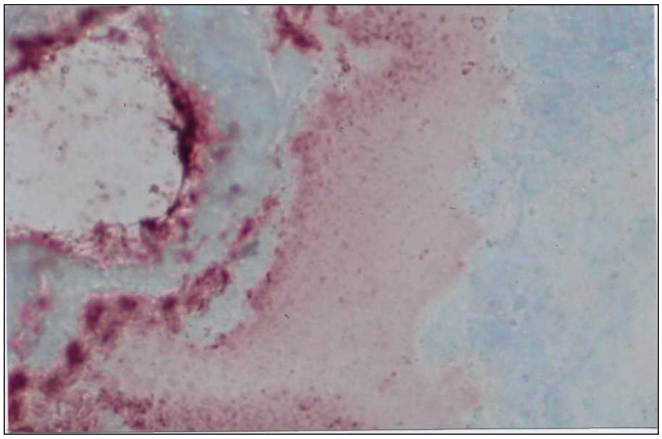

Fig. 5. Endometrial fragment with pronounced expression of TNF-α antigen (3 score) of a patient with preeclampsia of severe severity. Indirect streptavidin-peroxidase method for detecting TNF-α antigen expression. X – 200.

So, summarizing our studies of ultrastructural changes of endothelial cells in the microcirculatory channel of the fetoplacental complex during pregnancy with physiological course, it was found that endothelial cells completely retain their structure and function accordingly.

DISCUSSION

In preeclampsia in pregnant women, the morphological changes in the placenta are characterized by significant changes in the structural and functional activity of endothelial cells, a violation of the cell structure with the development of the initial stage of apoptosis. Studies have shown that in the syncytiotrophoblast epithelium, activation of lipid peroxidation occurs, as evidenced by the presence of the syncytiotrophoblastic membrane in the cytoplasm of the osmophilic structures. The latter accumulate in the cytoplasm from the formation of balloon structures .

Intercellular contacts are changing, there are signs of immune inflammation, a decrease in the number of fibrinoids, which confirms the growth of vascular permeability and the development of inflammatory response. Along with this there are ischemic and degenerative-dystrophic changes in the cells of syncytiotrofoblast. Violation of the rheological properties of the blood and damage to the parenchymal and stromal elements of the blood vessels of the microcirculation leads to fibrinoidal necrosis of the vascular wall of the villi, as a result of lymphocytic-macrophage infiltration there is a vasospasm, edema and thrombosis. Structural elements of syncytiotrophoblast are deeply affected and chorionic villus fibrosis develops.

In the background of morphologic changes in placenta, the level of IL-10 in pregnant women with preeclampsia of a mild degree does not differ significantly from that of pregnant women with a physiological course in the first half of pregnancy ($p>0.05$). In severe preeclampsia, IL-10 levels decreased twofold (11.25 ± 0.54 pg / ml ($r = 0.48$, $p <0.05$)).

The emphasis on the displacement of interleukin pools in the direction of cell-mediated inflammatory reaction suggests that in preeclampsia, the disorder of the microcirculation is an unfavorable background for the course of pregnancy. This is the result of a disturbance of the placenta function due to the inflammatory immune response by activating the maternal systemic inflammatory responses, which is manifested by the increased release of proinflammatory IL-2 and TNF -α interleukins.

The growth of IL-2 in severe preeclampsia is 1.89 times higher than that for a mild degree of gestosis ($p <0.05$). A direct correlation between the level of IL-2 and the preeclampsia development rate ($r = 0.71$, $p <0.05$) was revealed.

The level of TNF -α tends to grow only in the early stages of the development of preeclampsia, that is, with a mild and moderate manifestation of the course ($p <0,05$), and with severe preeclampsia, a sharp decrease in its level occurs, which obviously indicates the breakdown of certain parts of cellular immunity . The TNF-α antigen expression intensity index was 3 points, the rate of prevalence of the process reached $75.6 \pm 6.7\%$.

The revealed changes correlated with changes in the endometrium. In the endometrium, there was a focal-diffuse localization of the TNF-α antigen. The prevalence of the process ranged from 15 to 40%, with an average prevalence rate of $27.5 \pm 4.2\%$ (see Figure 4).

In addition, expression of the antigen of the factor of necrosis of the cells was detected in the endothelial cells of the microcirculatory bed and at the intensity of the coloration by the semi-quantitative method in balls was 1 point [7]. The process was of a focal nature and was within 10%. The highest intensity was recorded in the epithelium of the glands, in the cells of the macrophage-lymphocyte series, in the decidual cells, as well as in the cover uterine epithelium.

In pre-eclampsia, an increase in proinflammatory cytokine (TNF-α) is observed both in intensity and in the extent of prevalence (Fig. 5). Under the influence of TNF – α, not only the activity of the inflammatory process, but also the processes of thrombophilia increases, as TNF – α has a prothrombogenic effect. The realization of this effect is manifested by the

development of a sludge phenomenon and microthrombotic formation, which promotes the development of irreversible changes in the fetoplacental complex and causes fetal hypoxia. The growth of the expression level of the transforming growth factor indicates the immaturity of parenchymatous-stromal elements of the placenta and placental bed (see Figure 5).

At the same time, a sharp decline in the IL-10 with 23.66 ± 0.93 pg / ml, which occurs in the mild course, to 17.91 ± 0.43 pg / ml, corresponding to severe preeclampsia, indicates suppressor the activity of this IL in patients with preeclampsia of the lung and medium ($p < 0,05$). This fact also indicates the disruption of the functioning of the humoral suppressor of the immune system, which at the early stages of the development of precalampy provides protection of the fetus from the destructive effects of the mother's immune system under the influence of proinflammatory interleukins, prevents the suppression of compensatory immune mechanisms and, as a result, inhibits the development of preeclampsia. As a conclusion: the higher the IL-10 in the early stages of pregnancy, the course of it is more physiological, and vice versa, the lower is it, so the preeclampsia development increases.

In pre-eclampsia, IL-10 deficiency, coupled with TNF-α growth, stimulates the production of macrophage-proinflammatory protein, which binds to monocytes of blood and T lymphocytes, promotes changes in the ratio of Th l / Th 2 subpopulations inducing an immune response toward Th-1, i.e. in the direction of anti-suppressor, pro-inflammatory reversal of the local immune system. The growth of the expression level of the transforming growth factor confirms the immaturity of the parenchymatous and stromal elements of the placenta, especially in the area of syncytiotrophoblast and spiral vessels. The latter have an immature structure in which smooth muscle cells predominate.

Such vessels are capable of contraction and cause deterioration of blood supply to the placenta, which leads to endothelial dysfunction, placental ischemia and the development of ofinfarction. Violation of hemodynamics of placental blood flow causes placental hypertension and increased resistance of deciduals of arterial vessels.

CONCLUSIONS

Histo-ultrastructural analysis of placental changes and dysfunctional changes in the state of the cellular and humoral immunity links showed a correlation between the severity of pre-eclampsia and the nature of the structural and functional transformation of cell and tissue elements of the placenta during preeclampsia and the role of the imbalance of immune bonds of proinflammatory and anti-inflammatory interleukins as a factor blocker of proinflammatory interleukins inhibitor of a cell response and a stimulator of humoral response.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

INFLUENCE OF ANESTHESIA TYPE ON INTRAOCULAR PRESSURE DURING SPINE SURGERY IN PRONE POSITION

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ABSTRACT

The aim was to examine intraocular pressure (IOP) during lumbar spine surgery in PP under general vs spinal anesthesia and to compare it with volunteers in PP.

Materials and methods: We performed randomized prospective single institutional trial. Patients were operated in PP with fixation of 1-2 spinal segments. Patients of group I (n = 30) were operated under SA, group 2A (n = 25) – under TIVA (total intravenous anesthesia) with 45° head rotation (left eye was located lower, than right eye), group 2B (n=25) – under TIVA with no head rotation (both eyes were located on the same level). IOP was measured with Maklakov method before and after surgery. Volunteers (n = 20) were examined before and 90 minutes after lying in PP with 45° head rotation.

Results: In all patients and volunteers after lying in PP, we found that IOP have increased. In SA patients and in TIVA patients with no head rotation there was no difference between eyes. The most significant raise of IOP was found in the dependent eye of IIA group patients: it was higher than in volunteers and I group patients ($p < 0.01$), and IIB group patients ($p < 0.05$). In SA patients there was no difference in IOP comparing to volunteers.

Conclusions: IOP increased in PP in healthy people and patients under anesthesia (SA and TIVA). IOP in SA patients did not differ from volunteers. IOP increased superiorly in the dependent eye in TIVA patients.

KEY WORDS: intraocular pressure, spinal surgery, prone position, spinal anesthesia, intravenous anesthesia

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INTRODUCTION

Postoperative visual loss (POVL) is a quite rare, but devastating complication of surgeries performed in prone position which pathophysiology is not clearly understood [1]. It occurs in approximately 0.2% of surgeries in prone position [2]. The main mechanism of POVL is thought to be ischemic optic neuropathy in 89% of cases. Rarely, it can be a result of central retinal artery occlusion, cortical blindness, and posterior reversible encephalopathy [3]. Risk factors of POVL could be major surgeries in prone position, significant blood loss, intraoperative hypotension, diabetes mellitus, obesity, direct pressure on the eyeball, history of oncologic disease, and vasopressor requirement. Restoration potential of optic nerve is very poor, thus POVL in majority of cases is irreversible [4]. The mechanism of POVL is thought to be increasing of intraocular pressure and decreasing of perfusion pressure [5]. That is why the most effective prophylaxis of it is considered to be 10° head elevation, prevention of abdominal compression and direct eye pressure, hypovolemia, and hypotension.

Lumbar spine surgery can be performed under general and spinal anesthesia [6,7]. Spinal anesthesia has several advantages over general anesthesia, including lower blood loss, lower postoperative pain intensity, and lower incidence of postoperative nausea and vomiting. All currently available researches of intraocular pressure (IOP) during surgeries in prone position were performed in patients under general anesthesia [8]. Moreover, all cases of POVL were associated with general anesthesia [9].

THE AIM

Aim of the study was to examine changes of IOP during lumbar spine surgery in prone position under general vs spinal anesthesia and to compare them with healthy volunteers lying in prone position.

MATERIALS AND METHODS

After approval of local ethics committee we performed randomized prospective single institutional trial. Investigation was performed in SI "Sitenko Institute of Spine and Joint Pathology NAMS of Ukraine". We examined 20 healthy volunteers and 80 ASA I-II patients with degenerative lumbar spine diseases. Patients were operated in

Table 1. Intraocular pressure (mm Hg) in patients and healthy volunteers (M ± SD).

	Before surgery	Before surgery	After surgery	After surgery
	Right eye	Left eye	Right eye	Left eye
Group I	15.9±1.0	15.7±1.1	17.2±1.2*	17.8±1.5*
Group IIA	16.1±1.4	16.2±1.4	18.4±1.4*	21.2±1.9* **
Group IIB	15.9±1.4	16.0±1.3	18.8±1.7*	18.6±1.5*
Healthy volunteers	16.3±1.3	16.4±1.2	16.9±1.0	18,2±1.2* **

* $p < 0.05$ comparing to the first examination
** $p < 0.05$ comparing to the non-dependent eye

prone position with transpedicular screw fixation of 1-2 segments (PLIF – Posterior Lumbar Interbody Fusion). The length of surgery did not exceed 3 hours. Patients of group I (n = 30, 11 men, 19 women, age 46±12 years) were operated under spinal anesthesia at level L2-L3 or L3-L4 with bupivacaine heavy 0.5% – 3.5 ml. After local anesthetic injection patients lied in supine position during 10 min and then were turned into prone position. During the operation they could change the position of their head. Patients of group 2A (n = 25, 10 men, 15 women, age 45±13 years) were operated under TIVA (total intravenous anesthesia – propofol, fentanyl, rocuronium in standard dose). The head was rotated 45° (left eye was located lower, than right eye). Patients of group 2B (n=25, 11 men, 14 women, age 43±14 years) were operated under TIVA, but the position of the head was 90° (both eyes were located on the same level). Prone position was standard horizontal with pillows under pelvis that allowed abdomen to sag freely. IOP was examined with Maklakov method by single experienced examiner before surgery and immediately after it. Healthy volunteers (n = 20, 9 men , 11 women, age 49±12 years) were examined in supine position, and 90 minutes after lying in prone position. Their head was rotated 45°. Exclusion criteria were surgeries longer than 3 hours, blood loss more than 1 liter, history of ophthalmologic pathology. We used simple randomization. All patients signed an informed consent for participation.

Statistical analysis was performed with IBM SPSS 9.0. Normal distribution was confirmed with Colmogorov-Smirnov test. To evaluate the difference between groups Student t-test was used with Bonferoni correction.

Authors declare no conflict of interest.

RESULTS AND DISCUSSION

Before surgery there was no difference in IOP between groups of patients and healthy volunteers (Table 1).

It found that IOP have increased in patients of all groups and healthy volunteers after lying in prone position. In patients under spinal anesthesia (Group I) and in patients under TIVA with head 90 ° there was no difference between eyes. The most significant increasing of IOP was found in the dependent eye of IIA group patients: it was higher than in healthy volunteers and I group patients ($p < 0.01$), and IIB group patients ($p < 0.05$). Interestingly, that in patients of group I (spinal anesthesia) there was no significant difference comparing to healthy volunteers. Maximal measured level of IOP was 26 mm Hg. None of the patients had postoperative ophthalmologic complications.

Blood supply of the optic nerve depends on the perfusion pressure. Ocular perfusion pressure is the difference between mean arterial pressure on the level of eye and IOP or venous pressure (depending which one is bigger). Local arteriolar vasoconstriction can lead to ischemia regardless normal mean arterial pressure [10]. Like brain tissue, optic nerve blood supply has autoregulation, that can fail in up to 20% of healthy people [11].

Changes of IOP in different body positions in non-anesthetized people were investigated by Malihi M. [12]. It was shown, that IOP in dependent eye had been higher. In the paper Lee T, et al. [13] proved that in prone position in non-anesthetized people IOP increases more significantly in the eye, that is positioned lower. Lam A. showed, that in prone position in healthy volunteers IOP increases very quickly (during 8 minutes) [14]. Interestingly, they found much more significant changes in IOP in prone position, than we did. In supine position in patients under general anesthesia IOP usually decreases [15]. That is why POVL does not occur after surgery in supine position. Deniz M. et al. demonstrated increasing of IOP under general anesthesia in prone position. Their result was similar to our data according to a more significant changes in the dependent eye [16]. Authors recommend to use their results in patients with one-eye glaucoma: head position of 45 degrees with healthy eye lower than glaucoma eye in prone position may be beneficial for them.

According to our data we could recommend to use spinal anesthesia in patients with increased IOP as it has minimal influence on it.

CONCLUSIONS

1. Intraocular pressure increases in prone position in healthy people and patients under anesthesia (spinal and TIVA).
2. Changes of IOP in prone position in patients under spinal anesthesia did not differ from healthy volunteers.
3. The most significant changes of IOP were found in the dependent eye of patients under intravenous anesthesia.

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SOCIAL AND EMOTIONAL INTELLIGENCE AS A BASIS FOR COMMUNICATIVE RESOURCE FORMATION IN FAMILY CAREGIVERS OF PATIENTS WITH ENDOGENOUS MENTAL DISORDERS

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ABSTRACT

The aim of our study was to determine features of social and emotional intelligence in family caregivers of patients with endogenous mental disorders as a basis for communicative resource formation in family where a patient lives.

Materials and methods: A total of 273 family caregivers of patients with paranoid schizophrenia and bipolar disorder were involved into this survey under informed consent conditions. Control group included 55 mentally healthy respondents, in whose families there is no mentally sick family member. Emotional intelligence of family caregivers was measured using the psychodiagnostic test "EQ" by N. Hall. To assess level of social intelligence the J. Gilford and M. Sullivan test (in adaptation done by Mikhailova E.S.) was used. Values of $p < 0.05$ were considered significant.

Results: The study revealed that family caregivers of patients with schizophrenia and affective disorders demonstrate a decrease in emotional and social intelligence indicators, which creates significant obstacles for effective interpersonal family communication and for the harmonious functioning of a family, in which a mentally sick patient lives, in general. Difficulties of emotional regulation, emotional management, recognition of emotional states of other participants of communication related to the level of emotional and social intelligence of FC are factors, that complicate interpersonal relations in families of patients and reduce possibilities for psychosocial adaptation of all family members.

Conclusions: Revealed features should be taken into consideration when creating appropriate psycho-educational and psycho-corrective programs for family caregivers of patients with endogenous mental disorders.

KEY WORDS: family caregivers; endogenous mental disorders; coping behavior, psychocorrection

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INTRODUCTION

Interpersonal communication in families of patients with endogenous mental disorders (EMD) provides multiple tasks i.e. information exchange, coordination of efforts, aimed at treatment and social rehabilitation, effective redistribution of roles in family activities; ensures development of interpersonal relationships in changed family situation, better understanding of partner and self-understanding. Prerequisites for effective interpersonal communication in a family, where patient with EMD lives, include general principles of successful family communication organization, as well as communicative resource of communicative partners: activity and openness, sufficient self-disclosure in process of communication, congruence of communication; accuracy of non-verbal communication, consistency of verbal and non-verbal messages; sensitivity to partners pronouncements, ability to listen actively and to provide appropriate feedback; impartiality and empathy in accepting a mentally sick family member as a communicative partner; creating an atmosphere of psychological security; affiliation, mutual empathy and social support, respect for the partner, which is especially important under stress, frustration and problem conditions.

Family interpersonal communication is associated with significant emotional tension and intensity of interaction [1]. Family of a mentally sick patient functions in reality, which varies according to the dynamics of his mental condition, which requires constant adaptation and communicative flexibility from family caregivers in order to maintain family homeostasis and functionality of the entire family system [2]. At the same time, due to lack of proper training, insufficient knowledge of disease and psychological distress, caused by abrupt changes in mental status of a family member, family caregivers face significant strain, now widely known as "family burden", which negatively affects communication in whole family system. Disruption of interpersonal communication is one of the most pressuring problems of family functioning in families where a patient with EMD lives [3, 4, 5, 6].

Within the last two decades, the important direction in development of psychosocial treatment for endogenous mental disorders is the emphasis on positive effects of family participation in process of therapy [7]. Psychosocial interventions are introduced into system of therapeutic measures for mentally sick patients and are considered along with psychopharmacological influences as those

that significantly affect the final outcome of treatment in both inpatient and outpatient care [8]. Providing effective and flexible interpersonal communication in families of patients with EMD is a basis for ensuring the effectiveness of psychosocial therapy, and for psychoprophylaxis of mental disadaptation in family caregivers of patients with EMD.

Communicative resources (CR) are the basis, that not only provides effective coping with problem situations, but also enhances psychosocial adaptation. It is due to communicative psychological resources that an adaptive or non-adaptive communicative behavioral style is formed, which is based, first of all, on empathy, affiliation, locus of control, self-assessment of the individual. In a situation where the disease, on one hand, changes personality structure of a patient and leads to leveling and suppression of emotional sphere, and on the other hand – complicates adequate interpersonal communication both in family, and in society in general, study of basis for the main communicative resources in family caregivers of patients with EMD is the urgent problem. It is common knowledge that appearance of a mentally sick person in a family negatively affects quality of life of patient and his relatives. It results in changes of interpersonal relationships, states of high irritability and unmotivated aggression, or, on the contrary, states of very low energy potential of person with limited functional (emotional and volitional) capabilities. All these factors lead to disruption of psychological compensatory-adaptive reactions and conditions in relatives of patients with EMD, that creates prerequisites for psychosocial disadaptation in family caregivers [9, 10]. The extent to which family members are able to form an adequate relationship and adapt to new living conditions depends on their personal communicative resource, which is formed on the basis of their emotional and social intelligence. Low level of communicative resource leads to passive maladaptive behavior, social isolation and disintegration of personality.

In overcoming stressful situations and performing effective interpersonal communication in families where patients with EMD live, features of emotional and social intelligence play a decisive role. Thanks to the ability to recognize and understand own emotional states and emotional states of other people, the basis for providing effective and adaptive emotional and behavioral responses in a family-friendly environment occurs.

THE AIM

The aim of our study was to determine the features of social and emotional intelligence in family caregivers of patients with EMD as a basis for communicative resource formation in family where a patient with EMD lives.

MATERIALS AND METHODS

To achieve this aim, according to the principles of bioethics and medical ethics, under the informed consent, 243 family caregivers (FC) of patients, who were diagnosed with paranoid schizophrenia (PSch) – 168 persons (main group of family caregivers of patients with PSch, MGPSch) and patients with affective disorders (AD) – bipolar disorder, recurrent depressive disorder – 75 persons (main group of family caregivers of patients with AD, MGAD), were examined. The inclusion criteria were: informed consent for filling up the questionnaire, clinical and psychological examination and psychological testing, absence of previous appeals to a psychiatrist or narcologist, absence of craniocerebral trauma, 1-2 degree of affinity to a mentally sick family member. There were 49 wives, 25 husbands, 94 mothers/fathers of patients in MGPSch. There were 20 wives, 25 husbands, 30 – one of the parents of patients with AD. The subjects were in the age range from 26 to 63 years (average age: "wife" 37.5 ± 0.8 years, "husband" 42.3 ± 0.9 and "one of the parents" 60.3 ± 3.7 years). Most of the relatives (64.5%) had a sufficient educational qualification (secondary and higher education), 55.1% had a permanent job. 70.4% of the respondents of MGPSch and MGAD rated their living conditions as satisfactory, while 23.1% indicated an unsatisfactory financial state of family, which changed after the appearance of a patient with a mental disorder in family. Analysis of family relationships showed that presence of adequate relationships was stated by 19.5% of the MGPSch and 28.6% of the MGAD respondents, while 46.2% of MGPSch and 26.4% of MGAD respondents described their family relationships as conflict and emotionally unstable. Control group (CG) included 55 mentally healthy persons (35 female and 20 male), in whose families there was no mentally ill patient and who never sought help from a psychiatrist.

Emotional intelligence of family caregivers was measured using the psychodiagnostic test "EQ" by N. Hall. This questionnaire was designed to identify the ability to understand the interpersonal relationships that are represented in emotions, and to manage emotional sphere on the basis of decision making.

Hall's emotional intelligence test defines both general level of emotional intelligence and its particular determinants (emotional awareness, ability to control emotions, self-motivation, empathy, recognition of emotional states of other people). For the examination of FC of patients with EMD a full version of test was used. Obtained results were evaluated in 5 subtests, and composite score was also taken into account.

To assess the level of social intelligence the J. Gilford and M. Sullivan test (in adaptation done by Mikhailova E.S.) was used. Study of social intelligence through social intellect as a factor of psychosocial adaptation allows to estimate ability to understand and predict behavior of people in different life situations, to recognize the intentions, feelings and emotional states of person by verbal and nonverbal expression. This test defines both general level of social intelligence and its separate components (ability to predict consequences of behavior, to understand verbal and nonverbal behavior, to understand general picture of interpersonal interaction). Processing of the received data was performed by methods of mathematical statistics.

Table I. Indicators of emotional intelligence level in family caregivers of patients with endogenous mental disorders (mean score, M±m)

Indicators	Mean score (M ± m)		
	MGPSch	MGAD	CG
Emotional awareness	7,9±2,5**	12,5±1,5**	14,5±1,5
Managing emotions	3,2±1,7**	4,5±1,5**	15,5±1,5
Self-motivation	8,7±1,9**	11,4±1,9**	13,4±1,9
Empathy	5,5±2,3*	10,0±2,6*	12,0±2,6
Recognition of emotions of others	5,6±1,2*	12,3±1,5*	16,3±1,5
Composite score	36,4±2,9**	43,2±1,7**	70,5±3,7

Note: * - data are statistically reliable (P<0,05); ** - (P<0,001)

Table II. Indicators of emotional intelligence level in family caregivers of patients with paranoid schizophrenia depending on the duration of the disease in a mentally ill family member (mean score, M ± m)

Indicators	Mean score (M ± m)		
	MGPSch1 (under 4 years)	MGPSch2 (5-8 years)	MGPSch3 (8-12 years)
Emotional awareness	8,3±1,4**	9,5±1,5	6,5±2,5
Managing emotions	5,2±1,9	3,5±1,5	- 8,5±1,5**
Self-motivation	16,7±2,1*	10,2±1,9	10,4±1,9
Empathy	15,5±2,0*	10,0±2,6	- 1,0±2,6*
Recognition of emotions of others	5,6±1,1*	15,3±3,5*	- 6,3±1,5
Composite score	49,4±2,5**	33,2±2,7**	20,5±12,8**

Note: * - data are statistically reliable (P<0,05); ** - (P<0,001)

RESULTS AND DISCUSSION

One of the basic determinants of interpersonal interaction is emotional intelligence as a complex integral characteristic that provides recognition of own emotions and emotions of other people, as well as creates preconditions for managing emotions in situations of interpersonal communication.

Study of the emotional intelligence level in FC of patients with EMD (Table I) made it possible to find out some differences in main groups compared to CG. Received digital data by the scale of emotional awareness (MGPSch 7.9 ± 2.5 and MGAD 12.5 ± 1.5; CG 14.5 ± 1.5; P<0.001) suggest that family caregivers of patients with EMD are not always able to understand the meaning of both negative and positive emotions to gain knowledge about which decision is better to accept, that reflects reduced ability to perceive their own negative emotions as a source of understanding the need for changes in their lives, inability to feel and observe changes in their emotional states, to understand occurance of negative emotions as important indicator of life problems. They often experience significant intrapersonal difficulties due to diminished understanding of meaning of emotions for maintaining optimal well-being and control over life.

According to the emotional management subscale (MGPSch 3,2 ± 1,7 and MGAD 4,5 ± 1,5, CG 15,5 ± 1,5, P <0,001), the data indicate that respondents from both main groups compared to CG respondents, have significantly lower ability to keep calm and self-control under conditions of environmental pressure, to monitor their own feelings, as well as limited ability to cope with their feelings, when something frustrating happens; they are not entangled in negative emotions, quickly calmed down after unexpected complaints, easy to disconnect from negative experience. Thus, obtained data testify to decreased possibilities of emotional coping in presence of endogenous mental disorder in a family member of main groups respondents. Indicators of the self-motivation subscale (MGPSch 8.7 ± 1.9 and MGAD 11.4 ± 1.9, CG 13.4 ± 1.9, P <0.001) reflect decreased ability to remain calm and lumped if necessary, acting in accordance with the requirements of life in FC of patients with EMD. Unlike the respondents of the CG, they can not deliberately experience a wide range of positive emotions – such as positive mood, joy, inner growth and humor, creatively solve actual problems in spite of life obstacles, easily immerse in a state of tranquility, readiness and concentration by rejecting negative feelings when it comes to acting.

Empathy is an important resource to overcome problem and emotionally-stressful situations. Good empathy skills, based on altruistic motivation, can help individuals to overcome their own stress, and help in overcoming distress to others, who are in need of empathy. Underdevelopment of empathy skills contributes to formation of selfish motivation in an emotional response. Such motivation is aimed, first of all, at preserving own well-being and reducing own emotional stress [8, 9]. In family caregivers, in whose families a patient with EMD lives, the obtained results on the subscale of empathy (MGPSch 5.5 ± 2.3 and MGAD

Table III. Indicators of emotional intelligence level in family caregivers of patients with affective disorders depending on the duration of the disease in a mentally ill family member (mean score, M ± m)

Indicators	Mean score (M ± m)		
	MGAD1 (under 4 years)	MGAD 2 (5-8 years)	MGAD 3 (8-12 years)
Emotional awareness	2,3±3,4**	9,5±7,5	12,3±4,5**
Managing emotions	15,2±1,9	12,5±1,5	4,5±1,5*
Self-motivation	15,7±2,1	14,2±1,9	11,4±1,9
Empathy	15,5±4,5	20,0±2,6*	12,0±2,6
Recognition of emotions of others	15,6±1,1	15,3±3,5	12,3±1,5
Composite score	39,4±3,5	43,2±4,7	40,2±2,7

Note: * - data are statistically reliable (P<0,05); ** - (P<0,001)

Table IV. Indicators of social intelligence level in family caregivers of patients with endogenous mental disorders (mean score, M ± m)

Group	Subtest 1	Subtest 2	Subtest 3	Subtest 4	Composite indicator
MGPSch (n=168)	3,22±0,5*	2,97±1,1**	2,81±0,3**	3,12±0,7**	3,30±2,6**
MGAD (n=75)	3,49±0,9*	3,14±1,6**	3,08±1,1**	3,47±0,5**	3,66±0,2**
CG (n=55)	4,08±0,5	4,18±0,1	4,13±0,7	4,49±0,2	4,43±0,6

Note: * - data are statistically reliable (P<0,05); ** - (P<0,001)

Table V. Indicators of social intelligence level in family caregivers of patients with endogenous mental disorder, depending on the duration of the disease (mean score, M ± m)

Group	ST1	ST 2	ST 3	ST 4	CI
MGPSch1(n=57)	3,12±0,8*	3,1±1,1*	2,9±0,8*	3,45±0,9*	3,30±2,6**
MGPSch 2 (n=58)	2,72±0,5	2, 7±1,5	2,41±1,3	3,12±0,5	2,40±1,3
MGPSch 3 (n=53)	2,6±0,6	2,2±1,0	2,11±0,5	2,82±0,7*	2,44±1,1
MGAD1 (n=34)	3,41±0,9	3,12±1,6*	2,5±1,3**	2,87±0,5	2,74±0,8
MGAD 2 (n=22)	3,05±0,6	2,35±1,2	3,08±1,1**	3,37±0,6**	3,12±0,2**
MGAD 3 (n=19)	2,15±1,0*	2,09±1,0	2,1±1,5**	2,12±0,5	2,1±0,5*
CG (n=55)	4,08±0,5	4,18±0,1	4,13±0,7	4,49±0,2	4,43±0,6

Note: * - data are statistically reliable (P<0,05); ** - (P<0,001)

10.0 ± 2.6, CG 12.0 ± 2.6, P <0.05) show reduced ability to listen to the problems of other people and low sensitivity to emotional needs of others, limited understanding of emotions of the interlocutor, especially if they are not expressed directly, deteriorated recognition of emotions expressed by non-verbal means, low ability to feel the needs of the communicative partner, inability to adjust to other peoples emotions.

Indicators on the subscale of understanding the emotions of other people (MGPSch 5.6 ± 1.2 and MGAD 12.3 ± 1.5, CG 16.3 ± 1.5, P <0.05) show that FC of patients with paranoid schizophrenia and affective disorders, have diminished ability to calm the communicative partner, to respond adequately to the mood, intentions and desires of others, to understand the feelings of others, to be good advisers in person-to-person relationships, to help others use their intentions to achieve personal goals. Composite evaluation (MGPSch 36.4 ± 2.9 and MGAD 43.2 ± 1.7, CG 70.5 ± 3.7, P <0.001) suggests that, in general, family caregivers of patients with EMD have both decreased ability to recognition of their own emotions and emotions of others, and limited ability to manage their emotions in situations of interpersonal interaction.

Unlike general intelligence, emotional and social intelligence are metacognitive abilities aimed at integrating cognitive, interpersonal and emotional information for a holistic understanding of reality, and may change under the influence of psychosocial experience. While studying the dynamics of emotional intelligence scores in family caregivers of patients with EMD, the following patterns were found (Table II): family caregivers of patients with PSch are characterized with a decline in parameters of empathy (MGPSch1 15.5 ± 2.0; MGPSch2 10.0 ± 2, 6; MGPSch3 (- 1,0) ± 2,6; P <0,001), ability to recognize emotions of other people (MGPSch1 5,6 ± 1,1; MGPSch2 15,3 ± 3,5; MGPSch3 (- 6,3) ± 1.5, P <0.001), emotional control (MGPSch1 5.2 ± 1.9, MGPSch2 3.5 ± 1.5, MGPSch3 (-8.5) ± 1.5, P <0.001) and composite score of emotional intelligence (MGPSch1 49.4 ± 2.5; MGPSch2 33.2 ± 2.7; MGPSch3 20.5 ± 12.8; P <0.001) with duration of the disease in mentally ill family member. Such indicators can be interpreted as possible predictors of interpersonal

communicative deviations in families of patients with EMD, which may be explained by contradictions in verbal and nonverbal communication; communicative barriers; violation and distortion of emotional exchange; inability to tolerate negative emotions on the background of lack of empathy and emotional competence.

When comparing the emotional intelligence scores in family caregivers of patients with AD (Table III), higher rates of emotional awareness in FC group of patients with a duration of disorder from 8 to 12 years were revealed in comparison with respondents from groups with a shorter duration of disease in a family member (MGAD1 2.3 ± 3.4, MGAD2 9.5 ± 7.5, MGAD3 12.3 ± 4.5, P <0.001). The decrease in ability to manage their emotions with increasing duration of the disease (MGAD1 15.2 ± 1.9; MGAD2 12.5 ± 1.5; MGAD3 4.5 ± 1.5; P <0.05) was found, while the level of empathy was the highest in group of FC of patients with AD duration from 5 to 8 years (MGAD1 15.5 ± 4.5; MGAD2 20.0 ± 2.6; MGAD3 12.0 ± 2.6; P <0.05), which, presumably indicates a compensatory adaptation of FC to the presence of affective disorder in a family member.

Emotional competence and skills of effective emotional management, which include cognitive, affective and behavioral components, determine system of emotional and rational interpersonal understanding [11]. Social intelligence is considered as an additional factor contributing to effective interpersonal communication, which is the basis for the implementation of empathic-affiliation tendencies. Social intelligence manifests itself in ability to interact with other people, to assess correctly the psycho-emotional state and motivation of communication partners in situations of social interaction, to generalize social information and to predict development of interpersonal interactions. Study of social intelligence level in family caregivers of patients with EMD allowed to reveal certain significant differences in their behavioral and communicative patterns (Table IV). For the psychological testing of FC a full version of social intelligence test by Guildford was used. The results were evaluated in 4 subtests (ST, ST1 "story with completion", ST2 "expression groups", ST3 "non-verbal expression", ST4 "story with addition"), and also composite assessment was done.

Thus, according to ST1 – "story with completion" family caregivers had mediocre abilities in understanding behavior of a patient who is afflicted with PSch or AD under conditions of family functioning (MGPSch 3.22 ± 0.5 and MGAD 3.47 ± 0.9, CG 4.08 ± 0.5 P<0.05). They could only partially predict further actions of the patient based on the analysis of real situations of family and friendly communication, to understand the feeling of the patient, and on this basis, to determine the effectiveness of communication.

Results for ST1 allow us to conclude that family caregivers of patients with EMD may not always be able to clearly construct a strategy of their own behavior in order to achieve certain goals in treatment of patients. Their behavior may go beyond generally accepted norms, they incorrectly represent the results of their own actions and deeds of others.

According to ST2 – "expression groups", revealed scores allow us to think that family caregivers unlike respondents of CG, experience certain difficulties in assessment states and feelings by nonverbal features in process of interpersonal communication (MGPSch 2.97 ± 1.1 and MGAD 3.14 ± 1.6, CG 4.18 ± 0.1, P <0.001). Sensitivity to nonverbal expression greatly enhances the ability to understand others. The ability to read non-verbal signals of another person, to understand them and compare them with verbal ones, is the basis of intuition. When family caregivers of patients with PSch or AD build their behavioral line, they are more likely to focus on the isolated statements of the interlocutor rather than on display of their non-verbal communication. In their understanding, openness and benevolence are not always a guarantee of success in communication, they have reduced sensitivity to emotional states of other people and an incomplete understanding of their own emotional state in conditions of interpersonal interaction, which, undoubtedly, are direct factors that predetermine the dysfunctionality in interpersonal communication.

Performance in ST3 – "verbal expression" (MGPSch 2,8 ± 0,3 and MGAD 3,08 ± 1,1, CG 4,13 ± 0,7, P <0,001) indicates, that family caregivers, in whose families patients with affective disorders live, have decreased ability to assess the speech of interlocutor quickly and correctly in context of particular situation, to find the right tone for communication, they do not always correctly demonstrate role-based plasticity and understanding of verbal communication meaning. Results obtained in ST4 – "story with addition" (MGPSch 3,1 ± 0,7 and MGAD 3,47 ± 0,5; CG, respectively, 4,49 ± 0,2, P <0,001), show decreased ability in relatives of patients with EMD to analyze complex situations of interaction in interpersonal communication; they do not always correctly assess the adequacy of the situation for communication and do not fully anticipate how a person will behave in future.

Composite evaluation (MGPSch 3,30 ± 2,6 and MGPAD 3,66 ± 0,2; CG 4,43 ± 0,6; P <0,05) suggests that adaptive personality resources in family caregivers of patients with EMD have been reduced in interpersonal interaction, they experience difficulty in judgments about interlocutors, are less prone to contact, but more tactic and friendly in communicative interaction in comparison with the control group respondents. They experience difficulties in assessing the needs and intentions of communication, predicting their own behavior and the logic of future events.

In study of the dynamics of social and practical intelligence in family caregivers of patients with EMD, the following patterns were found (Table 5): in family caregivers of patients with PSch there was a decrease in social intelligence for all constructs in accordance with the increase in the duration of the disease in a mentally sick family member. This indicates a limited ability to solve social problems, to identify and correctly interpret situations of social interaction, which is probably due to the constant experience of ineffective interpersonal interaction within family system.

Instead, in study of constructs of social intelligence in family caregivers of patients with AD (Table V) compen-

satory increase in groups of FC of patients with duration of EMD up to 4 years and from 5 to 8 years (composite assessment MGAD1 2.74 ± 0.8; MGAD2 3.12 ± 0.2; MGAD3 2.1 ± 0.5; P <0.001), indicating that FC are attempting to adapt to the social situation by integrating both positive and negative social experience, attention to the situation context, desire to evaluate social situation objectively.

CONCLUSIONS

Revealed features should be taken into consideration while developing appropriate psycho-corrective systems for FC of patients with EMD aimed at development and improvement of family interpersonal communication quality in families of patients with EMD, in particular:

1) formation of the ability to predict consequences of social behavior, to understand manifestations of verbal and nonverbal communication, to navigate in the general picture of interpersonal interaction;
2) mastering skills of constructive emotional coping and self-management in conditions of stress caused by mental illness in a close family member;
3) creating conditions for expanding the range of emotional response and developing empathy and reflection in process of interpersonal interaction.

Consequently, family caregivers of patients with schizophrenia and affective disorders demonstrate a decrease in emotional and social intelligence indicators in all subtests, which creates significant obstacles both for effective interpersonal family communication and for the harmonious functioning of a family, in which a mentally sick patient lives, in general. Difficulties of emotional regulation, emotional management, recognition of emotional states of other participants of communication related to the level of emotional and social intelligence of FC are factors, that greatly complicate interpersonal relations in families of patients with EMD and reduce possibilities for psychosocial adaptation of all family members. Revealed features should be taken into consideration when creating appropriate psycho-educational and psycho-corrective programs for family caregivers of patients with endogenous mental disorders.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

GENITAL MUTILATION AS A CONSEQUENCE OF CRIMINAL OFFENCE AGAINST A PERSON

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ABSTRACT

The aim: The paper is aimed at creation of a procedure for determining the loss of any body organ or its functions, genital mutilation, as the signs of grievous bodily harm, penalty for which is stipulated by the Article 121 of the Criminal Code of Ukraine, as well as establishing the possibilities of legal setting of the concept of "genital mutilation".

Materials and methods: Over 100 criminal proceedings, involving forensic medical examination, under the Article 121 of the Criminal Code of Ukraine for the period from 2007 to 19.05.2019 have been studied. Common methods of research have been used, namely, the analysis and synthesis, statistical method, as well as own observations of the process of judicial examination of the above criminal proceedings, as well as individual interviews, survey of the victims and their family members in total of 39 people.

Results and conclusions: The findings of the investigation enabled detecting the gaps in the regulation of the procedure to define genital mutilation as a characteristic feature of grievous bodily harm. The ways of further improvement of the procedure of conducting expert studies of genital mutilation as a characteristic feature of bodily harm, which facilitates avoiding of errors in forensic medical and judicial practices have been found. The process of criminalization of the illegal conduct of surgical operations, resulted in mutilation of genitalia, must take place with the mandatory involvement of specialists in the field of practical medicine, as well as medical scientists specializing in sexopathology, gynecology, oncogynecology, forensic medicine in order to avoid errors in the lawmaking.

KEY WORDS: genital mutilation, genital injury, bodily harm, grievous bodily harm, signs of grievous bodily harm, surgical operations caused genital mutilation, the procedure of genital mutilation identification

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INTRODUCTION

Importantly, one of the main constitutional provisions is that human life and health is recognized as the highest value nationwide. That is why the Article 1 of the Criminal Code of Ukraine (hereinafter CC), developing the constitutional provisions, puts the human interests forward in the hierarchy of objects of criminal law. However, such legal foundation is not always effective in reduction of criminal activity related to offence against the life and health of a person. Thus, 38,274, 39,164 and 14,759 criminal offenses against life and health of a person were committed in 2017, 2018, and within three months of 2019, respectively. Among the offences against human life and health, special attention is given to the facts of offences causing grievous bodily harm (2058 cases in 2017; 2113 cases in 2018; 507 cases within three months of 2019), since in most cases they lead to the devastating damage to the health of a person, which in most cases resulted in disability of the victim.

Scientific researches and forensic medical practice carried out by domestic and foreign scientists have shown that, generally, the consequence of a criminal offence against victims' health is the loss of any body organ or its functions. Similarly, an examination of the adverse consequences of a criminal delinquency against the victim's health plays a significant legal role, which enables the authorities of the prejudicial inquiry and the court to assess correctly the actions of the defendants and establish the degree of their guilt in the presence of findings of properly conducted forensic examinations. In addition, the assessment of the severity of the damage to health caused by the criminal offence against the victim is a prerequisite for the implementation of a coherent program to protect the rights and interests of the victim. Such characteristic as the loss of any body organ or its functions, while determining the severity of bodily harm remains unclear to date (due to legal uncertainty) and the procedure for its determination is not well defined. Moreover, a new sign of grievous bodily harm as genital mutilation was defined in the CC of Ukraine in 11.01.2019. Consequently, it is uncertain whether the new interpretation is the equivalent of what existed before 11.01.2019, namely a loss of reproductive capacity, as a component of the concept "the loss of any body organ or its functions". However, the development of clear medical criteria for assessing bodily harm as grievous ones is relevant in contemporary forensic science.

The issues of legal regulation of setting punishment for causing bodily harm and the improvement of enforcement practice in this domain have been considered only fragmentarily in publications, devoted to criminal legal counteraction to bodily harm, problems on conducting forensic examinations in criminal proceedings, description of bodily harm. However, no scientific works related

to solving problems of imposing penalty for the offence causing grievous bodily harm, resulted in the loss of any body organ or its functions, genital mutilation, made jointly by the criminologists and medical researchers have never been conducted, resulted in the terminological confusion in current legislation on both criminal liability and public health care. At the same time, investigation shows that the issues of identifying grievous bodily harm that caused the loss of any body organ or its functions, genital mutilation are complex, that is, are related to both medical and legal domain.

THE AIM

The paper is aimed at creation of a procedure for determining the loss of any body organ or its functions, genital mutilation, as the signs of grievous bodily harm, penalty for which is stipulated by the Article 121 of the CC of Ukraine, as well as establishing the possibilities of legal setting of the concept of "genital mutilation".

MATERIALS AND METHODS

To elucidate the issues that arise in forensic medical and judicial practice in determining the loss of any body organ or its functions as a characteristic feature of grievous bodily harm, over 100 criminal proceedings under the Art.121 of the CC of Ukraine for the period from 2007 up to the present time (19.05.2019 inclusively), which involved forensic medical examination to identify cases for evaluation of victims on the loss of any body organ or its functions, have been studied. For this purpose, common methods of research have been used, namely, the analysis and synthesis, statistical method as well as own observations of the process of judicial examination of the above criminal proceedings. Additionally, for the purpose of analyzing the perception of victims of crime and their physical condition, individual interviews and survey have been conducted. Respondents of the investigation were directly victims and their family members in total of 39 people and 18 forensic experts. The survey was also conducted among judges, investigators and prosecutors (hereinafter – lawyers) in total of 129 people to elucidate their opinion on the need for amendments to legislative acts on the statement of signs of grievous bodily harm.

RESULTS

Section 1 of the Article 121 of the CC of Ukraine established a penalty for intentional grievous bodily harm, i.e., intentional bodily harm, which caused the loss of any body organ or its functions, genital mutilation, liable for imprisonment for a term of five to eight years. In the 2001 CC of Ukraine no substitution was made for the category "bodily harm" to "damage to health", which is the key one for the investigated group of criminal delinquencies. At the same time, as it was mentioned above, on January 11, 2019 in the CC of Ukraine a new sign of grievous bodily harm as genital mutilation was introduced. The paragraph 2.1.4 of the Rules of Forensic Medical Examination on defining the degree of bodily harm severity, approved by the Order of the Ministry of Health of Ukraine No. 6 as of January 17, 1995 (hereinafter referred to as the Rules), establishes that non-life-threatening injuries that "belong to the grievous ones according to the end result and the consequences are: the loss of any body organ or its function, namely, loss of vision, hearing, the tongue, arm, legs and reproductive ability" [1].

Apparently, while introducing the above amendments to the CC of Ukraine, the lawmaker did not even take care of the problem of defining the concept of "genital mutilation", whether the above concept is identical to definition of the loss of reproductive capacity, whether it is absorbed by it fully or partially, or the loss of reproductive capacity remains a part of the concept "the loss of any body organ or its function". Admittedly, the MOH of Ukraine had enough time (since the adoption of the Law of Ukraine "On the amendments to the Criminal and Criminal Procedural Codes of Ukraine to implement the provisions of the Council of Europe Convention on preventing and combating violence against women and domestic violence" as of 06.12.2017 before commencement on 11.01.2019) to make corrections to the Rules, but did not do so.

Subparagraph "d" of the Paragraph 2.1.4 of the Rules stipulates that the "loss of reproductive capacity should be understood as the loss of ability to coitus or fertilize, conceive and childbearing (childbirth)" [1]. These issues are resolved in accordance with the provisions of the Rules for conducting forensic expert examinations concerning the sexual status at the Bureau of Forensic Medical Examination, approved by the Order of the Ministry of Health of Ukraine No. 6 dated January 17, 1995 (hereinafter referred to as the Rules on Sexual Conditions). Thus, according to par. 2.5 of the Rules on Sexual Conditions, the expert examination for the establishment of the ability to sexual intercourse and to fertilization in females is conducted jointly by the commission in cases on defining the severity of bodily harm in resolving issues of the loss of the ability to reproduction. Consequently, the expert should establish: a) in determining the ability to intercourse: absence of congenital defects and malformations of female genitalia or absence of vaginitis; b) in determining the ability to fertilize (in view of age and anatomical and physiological characteristics): absence of gynecological diseases (endometritis, tumors, malposition of the uterus, etc.), hormonal disorders, chronic infections and intoxications, radiation effects, etc. [2].

An expert examination of the ability to sexual intercourse in males is carried out jointly by the commission with the participation of a sexopathologist and conducting: a) a survey of the subject; b) examination of the subject; c) laboratory tests; d) study of the medical documentation and, if necessary, the materials of the case. The findings on the ability of the examined person to sexual intercourse should be based on the aggregate data of the survey, examination, laboratory tests, medical records and case materials, taking

into account that the cause of sexual disorders could be severe exhausting general diseases, diseases of the central nervous system, inflammatory and other genital diseases, endocrine disorders, congenital defects (mutilation) of genitalia (e.g., prominent epi- or hypospadias), mechanical injury to the penis and the scrotum organs, and the presence of scarring and hardenings in the cavernous body, penis, lesions of the prostate (persistent lethargy, uneven tuberous surface, enlargement of one of the lobes, etc.) [2]. Thus, the Rules on Sexual Conditions consider the abnormality (mutilation) of genitalia as the reason for the loss of the ability of the subject to sexual intercourse (coitus).

The summary on the ability of the subject to fertilize should be based on the complex findings, considering the following: a) malformations of the penis (pronounced root or scrotal epi- and hypospadia) are not unconditional evidence of inability to fertilize; b) alterations in both testicles in the form of hardening and roughness (as a consequence of inflammatory process in the history) may be the cause of azoospermia, and such changes in one testicle only, while retaining the function of another, does not usually lead to loss of reproduction ability; c) detection of a bilateral hardening in the adnexa (as a consequence of inflammatory process or injury in the history), is usually an objective sign of inability to fertilize. The above cases can also occur in the unilateral lesion of the adnexa; d) pronounced scarring in the area of seminal vesicles and prostate (even in the absence of other lesions) can be a cause of azoospermia; e) from a forensic medical point of view, the presence of at least one normal spermatozoon in the ejaculate does not give grounds for assertion about the complete inability of the subject to fertilize. Notably, small number of spermatozoa (oligozoospermia) can only be indication of reduced, though not a complete, ability to fertilize [2].

It is obvious that the forensic and judicial practice of assessing the genital mutilation as the signs of bodily harm has not yet been formed, since little time have passed from the moment the relevant amendments to the CC of Ukraine were made; however, this cannot be a justification for developing the corresponding concept by the forensic medicine and criminal- legal science. Moreover, Article 38 of the Council of Europe Convention on Preventing and Combating Violence against Women and Domestic Violence (ETS No. 210, Istanbul Convention – hereinafter referred to as the Convention), recognized as the basic international document in the field of combating violence against women (Ukraine signed this Convention in 2011, though not ratified properly to date), provides for the prevention of female genital mutilation.

Article 38 of the Convention states that the "parties shall take the necessary legislative or other measures to ensure that the following intentional conducts are criminalized: a) excising, infibulating or performing any other mutilation to the whole or any part of a woman's labia majora, labia minora or clitoris; b) coercing or procuring a woman to undergo any of the acts listed in point a; c) inciting, coercing or procuring a girl to undergo any of the acts listed in point a" [3, p. 67].

However, the lawmaker, introducing genital mutilation as the signs of grievous bodily harm, meant the acts listed in the Convention, that is, the deliberate injury, or female genital injury, or female genital mutilation, or female circumcision, or included into the concept of the male genital mutilation? The provisions of the Convention refer only to clitoridectomy, excision and infibulation (Pharaoh's circumcision). That is, it is about partial or total removal of the external female genitalia. When completely removed, the surface is closed by sewing or even binding the legs for several weeks, which eventually closes the opening to the vagina and the opening of the urethra, leaving only a small opening (sometimes no more than a matchhead) for natural needs. Such operations on female genitalia are considered by the World Health Organization as mutilation (mutilatio; lat. "circumcision", "shortening"), which is recognized as a violation of human rights [4, p. 70].

The circumcision ritual is extremely harmful since it has negative outcomes, namely, 1) in more than 80% of cases it is carried out for girls from the first days of birth to 14-15 years old without any anesthesia and minimum hygiene; 2) it is mostly accompanied by severe bleeding, pain shock, wound infections, including tetanus and gangrene, or blood-borne infections such as HIV and Hepatitis B and C. Scarring, difficulty urinating, enuresis, menstrual disorders, multiple long lasting gynecological problems and other symptoms are the outcomes of mutilation. The UK National Health Service reports about a number of long-lasting health problems related to the outcomes of female genital mutilation, such as: 1) chronic vaginal and pelvic infections; 2) difficulty urinating, as well as persistent urinary tract infections; 3) renal failure; 4) dysfunction of the reproductive system, including infertility; 5) cysts and scar tissue formation; 6) complications during pregnancy and stillbirth; 7) pain during sexual contact and absence of a pleasant feeling; 8) psychological disorders, including low libido, depression and anxiety; 9) the need for further surgical intervention to open the vagina for sexual intercourse and childbirth [4, p. 70-71].

The World Health Organization reports about 18% of the female genital mutilation operations, carried out by healthcare professionals that raise serious concern about the fact that, actually, medical professionals violates the rights of women, undermining global efforts to eliminate such practices. Their participation provides this procedure not only with certain legitimacy, but also creates an idea of harmlessness or even health benefits [5].

However, the above considerations do not give us an answer to the question: what did lawmaker meant by the concept of "genital mutilation" as a sign of grievous bodily harm? The answer to this question is complicated by the fact that this sign was introduced into the Law of Ukraine "On the Amendments to the Criminal and Criminal Procedural Codes of Ukraine to implement the provisions of the Council of Europe Convention on Preventing and Combating Violence against Women and Domestic Violence" on 06.12.2017 and came into force on 11.01.2019. The limitation of the definition of genital mutilation only

in the context of the Convention reduces its meaningful load. Moreover, the lawmaker does not indicate in the disposition of section 1 of the Article 121 of the CC of Ukraine on female genital mutilation, which is referred to in the provisions of the above Convention. We can conclude from the English version of the Convention that this is not a case of mutilation as such, but about the conduct of surgical procedures leading to female genital mutilation.

Moreover, the study shows, that in Ukraine, genital mutilation can occur both in women and men as a result of the effect of various factors (not only due to surgical procedures leading to mutilation), including hard and blunt objects with flat limited surface of the collision (fist, feet, stick, hammer, head of an axe, stone; about 85% of cases), as well as traffic accidents (more than 6,2%), gunshot trauma (about 4,5%), sexual intercourse (1,3%), human teeth bite (2.5%), circumcision in the understanding of the Convention (0.001%).

Thus, in men, damage to the organs of the scrotum and the deep layers of the male external genital organs is recognized as characteristic of the transport, explosive and gunshot trauma, which are submucous hematomas, traumatic testicular dislocation, rapture of the testicular membranes and tissues, traumatic amputation of the penis and tear-contused wound. For example, a 33 year-old serviceman of the Armed Forces of Ukraine (hereinafter – the Armed Forces) K. have been exposed to numerous blows to the inguinal part of the body. He was reported about 6-7 minute-lasting loss of consciousness. The conservative treatment lasted for 15 days. In connection with growing scrotum swelling, surgical intervention was indicated as for the post-traumatic dropsy of right testicular (hydrocele). The postoperative period was complicated by post-traumatic orchiepididymitis (a combination of two diseases: inflammation of the testicle (orchitis) and epididymitis [6, pp. 144-147]. The patient recovered within 37 days, followed up with regular medical check-up due to the possible development of testicular atrophy and obstructive infertility. Moderate bodily harm was established on the basis of the duration of health disorder.

In the longer term, the complications of the follow up post-traumatic period, which obviously are manifested by the obstructive infertility, erectile dysfunction, can be seen in later life and when they occur, and the degree of bodily harm severity will be probably changed, which should be recorded by a forensic expert in the report. The issues of determining the degree of bodily harm severity in persons, who have been exposed to genital injury, judging by the resulting complications, occurred in the direct causal relationship with the trauma were not reflected even in the regulatory documents that regulate the procedure for determining the severity of bodily harm to a person. On the one hand, the injury itself is not so considerable (usually, bruising of the scrotum and hematoma of the testicles disappear within 3 weeks), but on the other hand a bruised area (concussion) of the testicle and its appendage affects the reproductive function. It is known that spermatogenous cells and epithelium are the most susceptible to injury, and, therefore, the disorder of spermatogenesis depends on the duration and degree of severity of circulatory disorders. Moreover, scrotal bruising, testicular hematoma can lead to fibrous degeneration of the tissue, and, when involved in the spermaduct process, to the persistent aspermia [7, p. 14-17].

DISCUSSION

Apparently, introduction of the sign "genital mutilation" to section 1 of the Art.121 of the CC of Ukraine becomes even more unclear, since such mutilation can be assessed as not only the grievous bodily harm. In almost 50% of the situations, injury is accompanied by acute and subacute post-traumatic epididymitis and orchiepididymitis, which is consistent with the study conducted by other scientists [8] who believe that traumatic testicular trauma is always a post-traumatic orchiepididymitis, and to a lesser extent, excretory-obstructive infertility [9]. Bodily harm are defined as the grievous ones on the basis of loss of body organ (3%); moderate bodily harm are defined on the basis of prolonged health disorders (3%); minor bodily harm are defined on the basis of short-term health disorders (34%); injuries were not considered as bodily harm(60%). Formal approach to determining the severity of bodily harm in the genital trauma does not take into account the possibility of the follow-up shocking reaction at the time of injury, and post-traumatic complications, the occurrence of which covers a sufficiently long period of time [7, p. 16].

V.A. Mozgova, after studying the provisions of the Convention and finding out the extent of the incidence rate of female genital mutilation (according to the UN, each year, parents put at risk 3,000,000 girls, estimating as more than 8,000 a day, for the purpose of female genital mutilation), has proposed the following amendments to the section 2 of the Criminal Code of Ukraine: "Article 121-1. Female genital mutilation:

1. A person is guilty of an offence if he/she abets a girl to excise, infibulate (re-infibulate) or performing any other mutilation to the whole or any part of her own labia majora, labia minora or clitoris and is liable for imprisonment for a term up to three years.

2. A person is guilty of an offence if he/she coerces a girl to excise, infibulate (reinfibulate) or performing any other mutilation to the whole or any part of her own labia majora, labia minora or clitoris and is liable for imprisonment for a term up to five years.

3. The acts under sections 1 or 2 of the Article committed by a close relative or a family member of a person shall be punishable by imprisonment for a term of three to five years.

4. The acts of excision, infibulation (reinfibulation) or performing any other mutilation to the whole or any part of girl's own labia majora, labia minora or clitoris regardless of her consent, shall be punishable by imprisonment for a term of five to ten years.

5. The act under section 4 of the Article, committed against a minor or incapacitated person, or repeatedly, or if

these acts have caused serious consequences, shall be punishable by imprisonment for a term of eight to twelve years.

6. The acts under section 4 of the Article, committed against a minor, or with respect to two or more persons, or close relatives or family member of a person, or if these actions caused death of a person, shall be punishable by imprisonment for a term of ten to fifteen years.

7. A citizen of Ukraine or a person permanently residing on the territory of Ukraine is guilty of an offence if he/she coerces a girl to be infibulated (reinfibulated) or otherwise mutilated the whole or any part of girl's own labia majora, labia minora or clitoris outside Ukraine and is liable for imprisonment for a term from three to five years" [4, p. 75–76].

In connection with the controversy of the introduction of the analyzed amendments to section 1 of the Art.121 of the CC of Ukraine the search for adaptation of the norms of the national legislation to the provisions of the above Convention is being updated. Therefore, the suggestion of V.A. Mozgova and other possible suggestions will require the establishment of a range of legal facts that may lead to the removal, infibulation or any other operation for the total or partial removal of the labia majora, labia minora or clitoris in specially accredited health care institutions. Importantly, such a range should include the legal fact associated with congenital defects of the labia and post-traumatic defects (after childbirth, road accidents, criminal offences, etc.) [4, p. 76].

Moreover, the statement of section 1 of the Art.121 of the CC of Ukraine as: "the intentional grievous bodily harm, that is, intentional bodily harm that is life-threatening at the time of infliction, or causing the loss of any body organ or its functions, genital mutilation ..." remains problematic. Apparently, intentional grievous bodily harm causes damage, which is manifested in one of its signs, and not vice versa. Based on the rules of the formal logic, at the statement of the loss of any body organ or its functions, etc., it is possible to assess the bodily harm as the grievous ones, but it is not the grievous bodily harm that causes the loss of any body organ or its functions.

CONCLUSIONS

1. Undoubtedly, the Council of Europe Convention on Preventing and Combating Violence against Women and Domestic Violence (ETS No. 210, Istanbul Convention) must be ratified by the Parliament of Ukraine properly. Ukraine must criminalize intentional illegal actions consisting in: 1) removal, infibulation or performing any other mutilation to the whole or any part of the labia majora, labia minora or clitoris; 2) coercing or inciting a woman to undergo the acts listed in the previous subparagraph; 3) inciting, coercing a girl to undergo acts listed in subparagraph 1.
2. The process of above criminalization must involve specialists in the field of practical medicine, as well as medical scientists in sexopathology, gynecology, oncogynecology, forensic medicine, and others, to avoid similar errors in the lawmaking, which occurred in amendments to the text of section 1 of the Art.121 of the CC of Ukraine. This is due to the fact that the lawmaker has actually distorted the content of the Convention by pointing to the genital mutilation (both male and female, as not indicated in the text) as a sign of the grievous bodily harm, but not as a consequence of illegal operation, resulting in mutilation of the genitalia. Therefore, we suggest the Rules should be supplemented with subparagraph 2.1.9 with the following statement: "Genital mutilation".

Under genital mutilation the consequences of the injury must be understood, whether or not they have led to loss of reproductive capacity, which correspond to at least one of the following: a) partial or total removal of the clitoris; b) partial or total removal of labia minora; c) partial or total removal of labia majora; d) partial or total removal of the penis; e) loss of the testicle; e) cicatrical deformity of the clitoris, or labia minora, or labia majora, or the penis, or the scrotum, the excision or diminishing of which is possible only by surgical intervention.

This criterion is not applicable: a) in cases of surgical intervention (in cicatrical deformation) prior the forensic medical examination to determine genital mutilation; b) for the abovementioned consequences after surgical interventions performed on medical reasons".

3. Section 1 of the Article 121 of the Criminal Code of Ukraine should be stated as follows: "infliction of intentional grievous bodily harm that is life-threatening at the time of infliction, or genital mutilation, the loss of any body organ or its functions, psychic illness or other health disorder, combined with persistent loss of capacity for work not less than one third, or termination of pregnancy or irreparable face defacement is liable for punishment…". And in the Rules, subparagraph 2.1.9 can be stated as follows: "Genital mutilation".

Genital mutilation is irreparable damage to the external genitalia, causing the loss of its natural appearance, whether it led or not to the loss of reproductive capacity. Under irreparable damage to the external genital organs it is necessary to understand such anatomical disintegration of tissues (lack of tissue part, cicatrical deformation, stamping-related deformation due to implantation of foreign bodies or substances, cicatricial changes), which severity does not decrease with time or under the influence of non-surgical means.

Genital mutilation involves the following irreparable damage: a) partial or total removal of the clitoris; b) partial or total removal of labia minora; c) partial or total removal of labia majora; d) partial or total removal of the penis; e) total removal of the scrotum; e) cicatrical deformation of the clitoris, or labia minora, or labia majora, or penis, or scrotum; e) deformation of the clitoris, or labia minora, or labia majora, or penis, or scrotum due to the implantation of foreign bodies or substances; g) stamping-related cicatricial changes in the clitoris, or labia minora, or labia majora, or penis, or scrotum.

Of note, if at least one of the items listed in subpar. a is anatomical disorder, occurred due to inadequate provision of medical care, the expert commission is entitled to consider this disorder as genital mutilation. Male circumcision, tattoo of the external genital organs does not refer to mutilation of the genital organs.

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ORIGINAL ARTICLE
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PREVALENCE OF THE GENERALIZED PERIODONTITIS IN PATIENTS WITH DIFFERENT GROUPS BLOOD, IN DEPENDING ON AGE AND PERIODONTAL BIOTYPE

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ABSTRACT

The aim of the study was to investigate the prevalence of generalized periodontitis depending on age and biotype of periodontium.

Materials and methods: We examined 855 males aged 20-55 years, who were divided into 2 groups: the main group – 570 surveyed with a generalized periodontitis, the comparison group – 285 dental healthy individuals.The diagnosis of generalized periodontitis was established by the classification Danilevsky M.F. (1994) and refined by using paraclinical indices. The periodontal biotype was determined using Hu-Friedy Colourvue Biotype Probe.

Results: As a result of the conducted researches was establish, in the carriers of blood group O (I) and A (II), developed forms of generalized periodontitis were found, on average, 2.7 times more often than the initial forms of the disease. Instead, at the representatives of B (III) and AB (IV) groups blood the frequency of initial GP – I degree was on average, 1.2 times greater than the prevalence of developed forms of generalized periodontitis. Also as a result of our researches, we found that the cluster A1 had 39.30% patients, cluster A2 was found at 28, 77%, and cluster B – 31.93% of the total number of patients with generalized periodontitis.

Conclusions: As a result of our research, it was found that in the carriers of the blood group O (I) and A (II), more advanced forms of generalized periodontitis were observed, which was confirmed by the presence of the biotype of the periodontal disease in the cluster A1 and A2.

KEY WORDS: generalized periodontitis, blood group, periodontal biotype

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INTRODUCTION

Epidemiological studies show a steadily high prevalence of periodontal disease already at young people [1,2]. According to most authors, the most common form of periodontal pathology in adolescents and children have gingivitis [3,4] . But with age, single frequency more severe lesions (periodontitis) and the number of teeth removed significantly increased [5]. When exploring out numerous papers on the leading role of local factors in the development of diseases of periodontal tissues, it can be noted their contradiction and the increasing need to use to explain some of the provisions role of endogenous factors [6]. In the study of pathogenetic mechanisms of development of inflammatory diseases of the periodontal, many hypotheses were formed, the main ones of which it is possible to consider immunological, neurogenic, vascular [7,8,9].

One drawback in the study of periodontal disease is the fact that many researchers trying to explain the apear of the pathological process pointing out only one reason[10,11]. However, in solving the problems of pathogenesis and diagnosis of periodontal diseases, it is necessary to agree with the point of view on the need for a systematic approach, the content of which is to ensure that each component of the body's biosystem is not interpreted as an independent formation [12].

THE AIM

Investigate prevalence generalized periodontitis in patients with different group affiliation blood depending on age and biotype of periodontium.

MATERIALS AND METHODS

The study was conducted in clinics in the city of Ternopil and Ternopil regional blood transfusion station in 2014 – 2016 years. We examined 855 males aged 20-55 years, who were divided into 2 groups. The main group consisted of 570 surveyed with a generalized periodontitis I- III severity who applied for periodontal help to our clinic. In the comparison group included 285 dental healthy individuals. Patients in both groups of the study were somatically healthy, which was confirmed by the absence of complaints, physical examination data and corroborated lack of non-communicable and infectious diseases, according to outpatients cards. The diagnosis of generalized periodontitis was established by the classification Danilevsky M.F. (1994) and refined by using paraclinical indices [13,14]. The periodontal biotype was determined using Hu-Friedy Colourvue Biotype Probe [15]. The obtained results were processed statistically using the software packages "Statgraphics" and "Statistica 6.0".

Table 1. Prevalence of generalized periodontitis in patients with a different blood group affiliation depending on age.

Blood groups	Degree GP	Age groups									
		20–24		25–29		30–34		35–44		45–55	
		abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
O (I)	GP I	21	43,75±7,16	15	31,25±6,69	12	25,00±6,25	–	–	–	–
	GP II	4	6,35±3,07	7	11,11±3,96	13	20,63±5,09°	18	28,58±5,69°,*	21	33,33±5,93°°,**
	GP III	–	–	4	5,47±2,66	11	15,07±4,18	26	35,62±5,60**	32	43,84±5,80**
A (II)	GP I	18	37,50±6,98	17	35,42±6,90	13	27,08±6,41	–	–	–	–
	GP II	3	4,62±2,60	9	13,85±4,28	15	23,07±5,22°°	16	24,61±5,34°°	22	33,85±5,87°°,*
	GP III	–	–	10	17,86±5,11▲	13	23,21±5,64	16	28,58±6,04	17	30,35±6,14
B (III)	GP I	25	38,46±6,03	15	23,07±5,22	16	24,62±5,34	9	13,85±4,28°°	–	–
	GP II	–	–	5	15,63±6,41	6	18,75±6,89	14	43,75±8,76	7	21,88±7,30*
	GP III	–	–	–	–	4	17,39±7,90	13	56,52±10,33	6	26,09±9,15■■
AB (IV)	GP I	23	43,40±6,80	11	20,75±5,70°	10	18,87±5,37°	9	16,98±5,15°°	–	–
	GP II	–	–	4	14,28±6,61	6	21,43±7,75	10	35,71±9,08	8	28,58±2,53
	GP III	–	–	–	–	6	37,50±12,10	6	37,50±12,10	4	25,00±10,82

Notes: 1. ° p <0.05; °° p <0.01 – a reliable difference in values with respect to data in patients 20-24 years old.
2. *p1<0,05; **p1<0,01 – a reliable difference in values with respect to data in patients 25-29 years old.
3. ■■p2<0,01 – a reliable difference in values with respect to data in patients 30-34 years old.
4. ▲ p3<0,05 – a reliable difference in values with respect to data in patients with 0 (I) blood group.

RESULTS AND DISCUSSION

According to the clinical examination in the 570 patients with generalized periodontitis (GP) with a different blood group affiliation, found that regardless of blood type, was reduced prevalence of early forms of GP (table 1). In patients with a blood group O (I) prevalence initial – I severity degree decreased from 43,75 ± 7,16% of 20-24 aged till to 25,00 ± 6,25% in patients aged 30-34 years was examined, p> 0,05, p_1> 0,05. and in carriers A(II) blood – from 37,50 ± 6,98% in the younger age group to 27,08 ± 6,41% in the age range 30-34, p> 0.05 p_1> 0.05, p_3> 0.05. The attention was attracted that in people with O(I) and A(II) blood groups aged 35-55 years initial generalized periodontitis – I severity degree are not diagnosed. In the patients with a blood group B(III) incidence of early forms of GP in the 20-24 year amounted to 38,46 ± 6,03%, p_3> 0.05 and significantly decreased to 13,85 ± 4,28%, p<0,01, p_3>0,05, in the patients aged 35-44 . In individuals with blood group AB(IV) prevalence GP initial – I severity degree in age intervals 20-24 years was significantly higher for the data in all age groups (p, p_1 <0.05; p2 <0.01). It should be noted that the carriers blood group B(III) and AB(IV) aged 45-55 years the original form of generalized periodontitis not diagnosed.

The prevalence of GP II degree of severity with age increased in all study groups, regardless of the blood group's [16,17].

Thus, in patients with O(I) blood group, aged 20-24 years, prevalence GP II degree severity was 6,35 ± 3,07% and gradually increasing, reached 20,63 ± 5,09% at age 30 -34 years p <0,05 and 28,58 ± 5,69% at the 35-44 aged patients p, p_1<0,05. In this case, the maximum incidence of GP II degree severity was found in the group examine aged 45-55 years – 33,85 ± 5,87%, p,p_1<0.01. In patients with A(II) blood group prevalence GP II degree severity increased from 4,62 ± 2,60% of 20-24 year examined to 23,07 ± 5,22% in the age range 30-34 years, p <0,01 and up to 24,61 ± 5,34% of aged group 35-44, p <0,01. The highest prevalence of the GP II degree severity was installed in the 45-55 aged group of patients – 33,85±5,87%, p<0,01, p_1<0,05 [18,19,20].

Attracted attention that the carriers B(III) and AB(IV) blood group with GP II degree severity in the younger age group (20-24 years) was not detected. At the age of 25-29 years, the prevalence of GP II degree severity was 15,63 ± 6,41% of representatives blood group B (III) and 14,28 ± 6,61% in patients with blood group AB(IV). The maximum spread GP II degree severity was in carriers B(III) and AB(IV) blood group was the age group 35-44 years – 43,75 ± 8,76% and 35,71 ± 9,08% respectively p_1> 0,05. In the 45-55 year old patient, representatives of B(III) and AB(IV) blood groups, the prevalence of GP II degree severity decreased and amounted to 21,88 ± 7,30%, p_1 <0,05 and 28,58 ± 8 53%, respectively.

Prevalence GP III degree severity in the study group was of the same trend that characterized its increase depending on age. However, this nosological unit not diagnosed at the age of 20-24 years in carriers of O(I) and A(II) blood groups and the representatives of B(III) and AB(IV) blood groups in the age range 20-29 years.

In people with O (I) blood group the frequency of detection of GP of the III degree of severity increased from 5.47 ± 2.66% at the age of 25-29 years to 35.62 ± 5.60% in 35-44 year-olds, p_2 <0 , 01, reaching the maximum values in the age group 45-55 years – 43,84 ± 5,80%, p_1 <0,01.

Table 2. Variations of periodontal biotypes depending from blood groups in patients with generalized periodontitis

Periodontal biotype	Generalized periodontitis (n=570)							
	O (I) (n=184)		A (II) (n=169)		B (III) (n=120)		AB (IV) (n=97)	
	abs.	%	abs.	%	abs.	%	abs.	%
Cluster A1-Thin S	81	44,02± ±3,64	78	46,15± ±3,83	32	26,67± ±4,04°	24	24,74± ±4,38°
Cluster A2-TS	53	28,80± ±3,38	47	27,81± ±3,45	40	33,33± ±4,30	33	34,02± ±4,81
Cluster B-TF	50	27,18± ±3,28	44	26,04± ±3,38	48	40,00± ±4,47°°	40	41,24± ±4,99°°

Notes: °p<0,01;
°°p<0,05 – reliably difference the data values in patients with GP with blood group 0 (I).

Attention was attracted to the fact that in the carriers group blood A (II) the prevalence of the GP of the III degree of severity increased with age: from 17,86 ± 5,11% in the examine 25-29 years old to 30,35 ± 6,14% in people aged 45-55 years, in the absence of statistical reliability in all age groups of the study (p_1, p_2> 0.05). In the representatives of B (III) and AB (IV), the blood groups of the GP of the III degree of gravity began to appear at the age of 30-34 years: in 17,39 ± 7,90% and 37,50 ± 12,10%, respectively. In the age group of 35-44 years old in patients with B (III), the blood group noted the maximum frequency of detection of this nosological unit: 56.52 ± 10.33% of the subjects with a decrease to 26.09 ± 9.15% examined at the age of 45- 55 years old, p_2 <0.05. Attention was attracted to the fact that in the blood carriers AV (IV) the prevalence of the GP of the III degree of severity at the age of 35-44 years was equal to the data of 30-34 year-old inspected (37,50 ± 12,10%, p_2> 0,05) and decreased to 25.00 ± 10.82 % of the patients in the age group 45-50 years.

Thus, as a result of the conducted researches, a significant dominance of the initial forms of generalized periodontitis has been established in young people with the prevalence of its developed forms in older age groups, regardless of the group membership of the blood. At the same time, in the carriers of blood group O (I) and A (II) blood groups, developed forms of generalized periodontitis were found, on average, 2.7 times more often (p <0,05) than the initial forms of the disease. Instead, at the representatives of B (III) and AB (IV) blood groups the frequency of initial GP – I degree was, on average, 1.2 times greater than the prevalence of developed forms of generalized periodontitis, p> 0.05.

According to literature data variant of the anatomy of the periodontal tissues can, with a high degree of probability, can predict option development and severity of periodontal pathological processes. Therefore, the first step was to determine the biotype of periodont in patients with GP with different blood groups.

As a result of our research, we established that the cluster A1 (thin – scalloped gingival biotype, ThinS) had 39.30% (224 patients), cluster A2 (thick – scalloped gingival biotype, TS) were found in 28.77% (164 inspected) and cluster B (thick – flat gingival biotype,TF) – 31.93% (182 people) of the total number of patients with generalized periodontitis (570 patients).

According to table 2, it was found that in patients with a GP of the O (I) blood group the cluster A1-Thin S was found in 44.02 ± 3.64% of the examined ones, which was comparable with the data in patients with GP, carriers A (II) blood groups (46,15 ± 3,83%, p> 0,05). At the same time, in patients with generalized periodontitis with B (III) and AB (IV) in the blood group, the periodontal A1 biotype was found reliably less (26.67 ± 4.04% and 24.74 ± 4.38%, p <0.01 , respectively). The average periodontal biotype (A2-TS) in the O (I) blood group was detected in 28,80 ± 3,38% of the examined ones, which did not differ from the data of the remaining groups of patients examined, patients with GP (p> 0,05). At the same time, it was noted that in the patients with generalized periodontitis with B (III) and AB (IV) blood group, the cluster B-TF was determined reliably more often (40,00 ± 4,47% and 41,24 ± 4,99 %, p <0.05, respectively) than in patients with GP from O (I) and A (II) in blood groups (27,18 ± 3,28% and 26,04 ± 3,38%, respectively).

Thus, as a result of the conducted researches, a significant dominance of the initial forms of generalized periodontitis has been established in young people with the prevalence of its developed forms in older age groups, regardless of the group membership of the blood. But attention was attracted that in carriers O (I) and A (II) blood groups, the prevalence of GP initial – I severity was significantly lower, and the frequency of detection of II-III severity GP was higher relative to the corresponding data in patients with B (III) and AB (IV) blood groups [20,21,22].

The obtained data about periodontal biotypes allow us to conclude that the carriers of O (I) and A (II) blood groups, suffering for GP was observed morphological inclination to the emergence and intensification of inflammatory and dystrophic changes in periodontium, due to the prevalence of gingiva biotypes of the cluster A1 and A2. At the same time, the carriers of B (III) and AB (IV) blood groups, with GP, determined the opposite tendency according to the cluster B-TF wich have a protective factor of predisposition to the inflammatory diseases of periodontal tissues [23,24].

CONCLUSIONS

This study clarifies and complements the data on the incidence of generalized lesions of periodontal tissues in people of all ages, depending on the blood group by a system AB0. Monitoring of the cluster affiliation of periodontal biotype can serve as an early marker for the diagnosis of periodontal tissue diseases, will allow for the correction and optimization of therapeutic measures for this contingent of patients.

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ORIGINAL ARTICLE
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TIME COURSE OF ENDOTHELIAL DYSFUNCTION AND ATHEROTHROMBOSIS MARKERS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITH ST SEGMENT ELEVATION AND TYPE 2 DIABETES MELLITUS DEPENDING ON REPERFUSION THERAPY APPROACH

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ABSTRACT

The aim: To evaluate the levels of plasminogen activator type 1 inhibitor, asymmetric dimethylarginine and endothelial nitric oxide synthase on day 10-14 in patients, depending on the presence or absence of concomitant type 2 diabetes and the type of reperfusion therapy.

Materials and methods: The study involved 130 patients with acute myocardial infarction, divided into 2 groups: Group 1 consisted of patients with acute myocardial infarction with type 2 diabetes mellitus (n = 73), Group 2 comprised patients with acute type 2 diabetes mellitus (n = 57). The quantitative content of IAP-1 was determined by enzyme-linked immunosorbent assay using a commercial test system manufactured by Technoclone PAI-1 ELISA Kit (Austria), NOS – Enzyme-Linked Immunosorbent Assay (ELISA) Kit for Nitric Oxide Synthase Endothelial (NOS) ADMA ELISA Kit (Austria).

Results and conclusions: Percutaneous coronary intervention contributes to a significant reduction in the content of ADMA, which is a marker of endothelial dysfunction and increase NOS on the 10-14th day of acute myocardial infarction compared with standard therapy. During PCI, the level of IAP-1 did not significantly change in the time course of treatment due to post-inflammatory and post-traumatic activation of platelets in the vascular wall.

KEY WORDS: acute myocardial infarction, type 2 diabetes mellitus, endothelial dysfunction, asymmetric dimethylarginine, plasminogen activator inhibitor type 1, percutaneous coronary intervention

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INTRODUCTION

Coronary heart disease (CHD) is the leading disease in the circulatory system [1]: in Europe, it accounts for about 38% of the prevalence rate and 19.5% of overall mortality [2]. Despite some advances in the study of pathogenesis, diagnosis and especially treatment, including the widespread use of various methods of revascularization, disability and lethality in this disease remains extremely high. Acute myocardial infarction remains the leading cause of disability leading in mortality worldwide.

It is impossible to underestimate the impact of type 2 diabetes on the incidence of acute myocardial infarction, which is higher than the average in the population and the prognosis of life in such patients is much worse than that in patients without disorders of carbohydrate metabolism [3].

Endothelial dysfunction is a crucial factor among many pathogenic mechanisms of vascular injury in ischemic heart disease and type 2 diabetes [4, 5], in which inflammation and endothelial dysfunction play an important role in the development of MI [6,7,8,9].

According to current data, in addition to these factors, a major role in the development of cardiovascular complications of diabetes belongs to the reduction of fibrinolytic activity [10,11], which is associated with an increase in the concentration and activity of plasminogen activator inhibitor type 1 (IAP-1) [12].

Inhibition of fibrinolysis is due to a number of factors that are able to directly bind plasmin or retard plasminogen activation. One of the main inhibitors of fibrinolysis is IAP-1 (also called endothelial type of inhibitor), which is constantly produced and secreted by endothelial cells.

Scientific literature suggests that plasminogen activator inhibitor type 1 is involved not only in the metabolic processes of atherosclerosis, causing thrombosis, but also in the development of type 2 diabetes [13,14]. However, due to the fact that there are conflicting data on the concentration of this marker in the time course of treatment [15], in our study we analyzed the time course of IAP-1 levels in the presence of a combined pathology of acute myocardial infarction and type 2 diabetes, depending on therapeutic approach.

Endothelial dysfunction is considered a key link in the pathogenesis of microvascular complications of diabetes. Disruption of endothelium-dependent vasodilation is one of the main effects arising from the accumulation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase – NOS, which is formed in the process of methylation of the amino acid L-17].

ADMA is currently being studied as a marker of endothelial dysfunction [18].

To date, percutaneous coronary intervention (PCI) has been widely implemented in the practice of endovascular ACS treatment, which is a lifesaving procedure and the best reperfusion method for acute myocardial infarction with ST segment elevation [19]. Numerous factors contributing to the development of acute coronary events after stenting have been extensively discussed in the literature [20, 21].

THE AIM

To assess the time course of levels of plasminogen activator inhibitor type 1, asymmetric dimethylarginine and endothelial nitric oxide synthase on day 10-14 in patients, depending on the presence or absence of concomitant type 2 diabetes mellitus and the selected therapeutic approach, namely percutaneous coronary intervention (coronary stenting) or low molecular weight heparin therapy in combination with dual antiplatelet therapy.

MATERIALS AND METHODS

The study involved 130 patients with acute myocardial infarction who were hospitalized in the infarction department of Kharkiv City Clinical Hospital No. 27, Intensive Care Unit and Cardiology Department of the municipal non-profit enterprise of Kharkiv Regional Council "Regional Clinical Hospital" and the Department of Interventional Cardiology of V.T. Zaitsev Institute of General and Urgent Surgery of the Institute of National Academy of Medical Sciences of Ukraine. All patients were divided into 2 groups: Group 1 included patients with acute myocardial infarction with type 2 diabetes mellitus (n = 73), Group 2 comprised patients with acute type 2 diabetes mellitus (n = 57). Group 1 included 43 men (41.7%) and 30 women (58.3%); Group 2 included 43 men (70.9%) and 14 women (29.1%). The mean age of patients in Group 1 was 62.73 ± 1.40 years, and in Group 2 63.98 ± 1.47 years. The control group consisted of 20 practically healthy individuals, whose average age was 60.85 ± 0.17 years.

The quantitative content of IAP-1 was determined by enzyme-linked immunosorbent assay using a commercial test system manufactured by Technoclone PAI-1 ELISA Kit (Austria), NOS by Enzyme-Linked Immunosorbent Assay (ELISA) Kit for Nitric Oxide Synthase Endothelial (NOS), Asymmetric Dimethylarginine – Immunodiagnostik ADMA ELISA Kit (Austria).

Four subgroups of patients were formed for comparative evaluation of the therapeutic effect of combination therapy in acute myocardial infarction: 1st subgroup of patients with acute type 2 diabetes mellitus who underwent PCI (coronary stenting) (n = 58); 2nd subgroup of patients with AMI with type 2 diabetes, who underwent standard anticoagulation therapy with enoxaparin/fondaparinux in combination with dual antiplatelet therapy (n = 15); 3rd subgroup of patients with AMI without type 2 diabetes who underwent PCI (n = 40); 4th subgroup (n = 17) comprised patients with AMI without type 2 diabetes who underwent standard anticoagulation therapy.

Mathematical computer processing of the results was performed using the software Statistica 6.0 (StaSoft Inc, USA). We calculated: mean (M), variance, standard deviation, median (m), probability and significance level (p). For comparative analysis of the samples with normal distribution, the significance of the differences was confirmed using the Student's t test (t) and a standard correlation analysis program with the calculation of arithmetic mean values: M + m, σ, probability and confidence level (p). Non-parametric Mann-Whitney test was used for pairwise comparisons of the means in the groups. Non-parametric paired Wilcoxon test was used in the analysis of samples not subject to the Gaussian distribution laws.

RESULTS AND DISCUSSION

Patients with concomitant type 2 diabetes (Table I) on day 1 of acute myocardial infarction with ST segment elevation were found to have significantly higher levels of IAP-1 compared with patients without concomitant diabetes mellitus (62.38 ± 1.83 ng/ml) and 50.99 ± 2.01 ng/ml; 66.72 ± 1.63 ng/ml and 51.11 ± 3.03 ng/ml, respectively; p <0.001). These findings suggest that patients on day 1 of AMI in conditions of acute occlusion of the coronary artery secondary to insulin resistance have a greater tendency to thrombosis, compared with patients without disorders of carbohydrate metabolism.

On day 10-14 of AMI in the group of patients with type 2 diabetes, there was no significant decrease in the level of IAP-1 compared with the results on day 1, as with stenting (62.38 ± 1.83 ng/ml and 61.75 ± 1.81 ng/ml, respectively; p>0.05), and when using standard antiplatelet therapy (66.72 ± 1.63 ng/ml and 60.78 ± 0.90 ng/ml, respectively; p >0.05), which may indicate a prolonged risk of thrombus formation secondary to insulin resistance.

In patients with type 2 diabetes mellitus on day 10-14 of treatment, there was a significant decrease in serum IAP-1 in patients both with PCI (50.99 ± 2.01 ng/ml and 45.55 ± 1.88 ng/ml; p <0.05) and when using standard antithrombotic therapy (51.11 ± 3.03 ng/ml and 42.63 ± 1.94 ng/ml; p <0.05).

According to the results of our study (Table II), significantly higher levels of ADMA were observed in patients with concomitant type 2 diabetes mellitus on day 1, regardless of treatment tactics (1.031 ± 0.038 μmol/l and 0.57 ± 0.025 μmol/l; 1.063 ± 0.06 μmol/l and 0.62 ± 0.029 μmol/l, respectively; p <0.001).

In the study of ADMA on day 10-14 of AMI there was a significant decrease in the level of ADMA in comparison with the results on day 1 of AMI, both with stenting and

Table I. Time course of IAP-1 levels on day 1 and day 10-14 of acute myocardial infarction, depending on the chosen therapeutic approach

IAP-1 level, ng/ml	Patients with acute myocardial infarction with concomitant type 2 diabetes, n=73		Patients with acute myocardial infarction, n=57		p
	With stenting, n= 58	Without stenting, n= 15	With stenting, n= 40	Without stenting, n= 17	
	1	2	3	4	
Day 1 of AMI	62.38±1.83*	66.72±1.63**	50.99±2.01^	51.11±3.03"	p_{1-2}>0.05 p_{3-4}>0.05 p_{1-3}<0.001 p_{2-4}<0.001
Day 10-14 of AMI	61.75±1.81*	60.78±0.90**	45.55±1.88^	42.63±1.94"	p_{1-2}>0.05 p_{3-4}>0.05 p_{1-3}<0.001 p_{2-4}<0.001

Note: *, ** – statistical significance of differences p> 0.05; ^,"- statistical significance of differences p <0.05.

Table II. Time course of ADMA level on day 1 and 10-14 of acute myocardial infarction depending on the chosen therapeutic approach

ADMA level, μmol/l	Patients with acute myocardial infarction with concomitant type 2 diabetes, n=73		Patients with acute myocardial infarction, n=57		p
	With stenting, n= 58	Without stenting, n= 15	With stenting, n= 40	Without stenting, n= 17	
	1	2	3	4	
Day 1 of AMI	1.03±0.04*	1.06±0.06**	0.57±0.03×	0.62±0.02^	p_{1-2}>0.05 p_{3-4}>0.05 p_{1-3}<0.001 p_{2-4}<0.001
Day 10-14-й of AMI	0.84±0.03*	0.87±0.05**	0.51±0.03×	0.45±0.04^	p_{1-2}>0.05 p_{3-4}>0.05 p_{1-3}<0.001 p_{2-4}<0.05

Note:*, **, ×, ^ – statistical significance of differences, p<0.05.

Table III. Time course of the level of NOS on day 1 and 10-14 of acute myocardial infarction, depending on the chosen therapeutic approach

NOS level, ng/ml	Patients with acute myocardial infarction with concomitant type 2 diabetes, n=73		Patients with acute myocardial infarction, n=57		p
	With stenting, n= 58	Without stenting, n= 15	With stenting, n= 40	Without stenting, n= 17	
	1	2	3	4	
Day 1 of AMI	3.10±0.16*	3.22±0.35**	4.26±0.13 ×	4.15±0.23^	p_{1-2}>0.05 p_{3-4}>0.05 p_{1-3}<0.001 p_{2-4}<0.05
Day 10-14 of AMI	4.81±0.15*	4.61±0.19**	7.42±0.57 ×	9.14±1.04^	p_{1-2}>0.05 p_{3-4}>0.05 p_{1-3}<0.001 p_{2-4}<0.001

Note:*, **, ×, ^ – Statistical significance of differences, p<0.05.

without stenting of coronary arteries in groups of patients with type 2 diabetes (1.03 ± 0.04 and 0.84 ± 0.03 μmol/l; 1.06 ± 0.06 and 0.87 ± 0.05 μmol/l), and without (0.57 ± 0.03 and 0.51 ± 0.03 μmol/l; 0.62 ± 0.02 and 0.45 ± 0.04 μmol/l, respectively; p <0.05), while in patients with type 2 diabetes, ADMA levels remained significantly higher than in patients without type 2 diabetes regardless of therapeutic approach (0.84 ± 0.03 μmol / l; 0.87 ± 0.05 μmol / l; 0.51 ± 0.03 μmol/l; 0.45 ± 0.04 μmol/l respectively; p1-2,3-4> 0.05, p1-3 <0.001, p2-4 <0.05).

The data obtained indicate a positive effect of CA stenting and standard therapy on the time course of asymmetric dimethylarginine in these categories of patients, which is confirmed by a significant decrease in ADMA in the time course of AMI within 10-14 days after treatment.

The value of endothelial oxidative stress in the progression of ischemic coronary artery disease in acute myocardial infarction cannot be underestimated, so we analyzed the levels of endothelial nitric oxide synthase in this cohort of patients.

Our study showed (Table III) that patients on day 1 of AMI with concomitant type 2 diabetes had significantly lower NOS levels compared with patients without type 2 diabetes, as in subgroups with CA stenting (3.10 ± 0.16 ng/ml and 4.26 ± 0.13 ng/ml, respectively; $p<0.001$) and during standard anticoagulant and dual antiplatelet therapy (3.22 ± 0.35 ng/ml and 4.15±0.23 ng/ml, respectively; $p<0.05$), which indicates the suppression of the anti-ischemic barrier of the endothelium secondary to insulin resistance observed in the majority of patients with type 2 diabetes.

On day10-14 of acute myocardial infarction there was a significant increase in the level of NOS, regardless of the therapeutic approach, both in patients with concomitant type 2 diabetes and without diabetes, but in patients with type 2 diabetes less intense (4.82 ± 0.31 and 4.61 ± 0.42 ng/ml; 7.42 ± 0.57 ng/ml; 9.14 ± 1.04 ng/ml, respectively; p1-2> 0.05, p3-4 > 0.05, p1-3.2-4 <0.001).

This indicates a gradual recovery of endothelial function secondary to the positive effects of treatment, especially in patients without disorders of carbohydrate metabolism.

The restoration of endothelial function in the early post-infarction period in patients with type 2 diabetes and without it is evidenced by a statistically significant decrease in the marker of endothelial dysfunction of ADMA and an increase in the concentration of endothelial nitric oxide synthase by the 10-14th day of AMI in all the examined patients both with CA stenting, as well as following standard therapy and the presence of concomitant pathology in the form of type 2 diabetes. In patients with type 2 diabetes mellitus ADMA levels remained significantly higher than in patients without type 2 diabetes mellitus, irrespective of therapeutic approach indicating a decrease, but a significant excess of the normative values of endothelial dysfunction processes, compared with patients with AMI without type 2 diabetes mellitus in early post-infarction period.

Regarding the level of the thrombus formation marker IAP-1, in patients with AMI with ST segment elevation, an increase in the concentration of IAP-1 in both groups of patients was detected, and the presence of type 2 diabetes defined higher values of this indicator during the first day after ST elevation. At the same time, in the early recovery period of AMI with ST segment elevation in patients without disorders of carbohydrate metabolism, there was a significant decrease in the level of IAP-1, indicating a decrease in the inhibitory effect of IAP-1 and improved fibrinolytic activity of the blood secondary to treatment. Patients with type 2 diabetes in the early recovery period after AMI (10-14 days after treatment) had persistently ($p <0.001$) higher level of IAP-1. The obtained data also suggest that patients with acute myocardial infarction with ST segment elevation within 10-14 days after PCI have a prolonged nature of increased serum IAP-1 concentration due to the inflammatory consequences of post-procedural activation of platelets and decreased function of endogenic thrombolytic system.

CONCLUSIONS

1. In patients with acute myocardial infarction with type 2 diabetes, percutaneous coronary intervention contributes to a significant decrease in asymmetric dimethylarginine content and an increase in NOS on the 10-14th day of acute myocardial infarction, indicating a positive effect of the performed revascularization.
2. Percutaneous coronary intervention in patients with AMI with ST-segment elevation and type 2 diabetes was not accompanied by a significant decrease in IAP-1 level by day 10-14 of AMI, which suggests a prolonged nature of increased serum IAP-1 concentration due to inflammatory consequences of post-procedural platelet activation and decreased endogenous thrombolytic system functioning.

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REMODELING OF THE DUCT SYSTEM OF THE RAT SUBMANDIBULAR SALIVARY GLANDS IN CHRONIC ETHANOL INTOXICATION

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ABSTRACT

The aim: To determine the dynamics of changes in metric parameters of the duct system of rat submandibular glands in normal conditions and chronic ethanol intoxication.

Materials and methods: 50 albino outbred rats were involved in the experiment. 10 animals were assigned in the control group, 40 animals – in the experimental group. Animals were sacrificed on 5, 9, 12 and 30 days by overdose of thiopental anesthesia. Pieces of the submandibular glands were embedded into epon-812 according to the conventional technique.

Results: On day 5 of the experiment the lumen diameter of intercalated duct reduced by 9,15 % ($p < 0,05$). The lumen diameter of the striated ducts was by 5,29 % significantly greater than the values in controls ($p < 0,05$). The lumen diameter of the granular ducts reduced by 2,45 % ($p < 0,05$). On day 30 of the experiment the height of the epithelial cells of the intercalated ducts was by 8,47 % significantly less ($p < 0,05$), the height of the epithelial cells of the striated ducts was by 12,27 % less ($p < 0,05$) and the height of the epithelial cells of the granular ducts was by 11,96 % less ($p < 0,05$) than the values in controls.

Conclusions: No recovery of parameters occurs by day 30 of the experiment, indicating the depletion of the secretory epithelium of the duct system, due to dystrophic changes caused by vascular disorder in the microvasculature.

KEY WORDS: salivary gland, duct, rats, ethanol

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INTRODUCTION

Major salivary glands are located outside the oral cavity, and enter it via the excretory ducts [1]. The system of the excretory ducts of the salivary gland lobes consists of intercalated, striated and collecting excretory ducts [1, 2]. Intercalated ducts located between the acini and striated ducts. They are lined by the low-cuboidal or flattened epithelial cells with a poorly expressed organelle apparatus. Epithelial cells with clear cytoplasm are characterized by the presence of dense granules with mucoid secretion on the apical part of the cell [3]. According to the foreign publications, these granules are more often found in the cells of the ducts, adjacent to the acini. The outer layer of cells in the intercalated ducts is formed by the fusiform myoepithelial cells. Noteworthy, intercalated ducts contain cambial elements of the acini and the system of excretory dusts [4]. These elements are differentiated into the glandular cells or duct cells, enabling regeneration of above mentioned parts of the glands [5].

Striated ducts are presented in the form of wide tubules, lined by oxyphilic highly prismatic cells with orbicular centric nuclei. Apical part of the cell protrudes into the wide lumen and lined by short microvilli. It accumulates the secretory granules, which mainly contain kallikrein [6]. G.A. Yeroshenko reports that it is precisely these cells that are involved in the development of a number of substances and growth factors that are secreted by human salivary glands. Importantly, in rodents, and above all, in rats, these and other biologically active substances are produced more actively than in humans [7]. In the lobules of the rat submandibular glands, secretory granules in the epithelial cells of the granular ducts contain kallikrein and ensure local mechanisms for regulating blood flow to the vascular system of the salivary glands [8]. Salivary glands produce the saliva, which plays a significant role in maintaining the homeostasis of the oral cavity [9, 10]. Recently, the interest of researchers in the study of the patterns of the salivary glands response to various stimuli has increased significantly, which is due to the diagnostic value of saliva as a highly informative object for the clinical assessment of the state of the overall health [11].

The World Health Organization reports about 2.5 million alcohol-related deaths worldwide, accounting for 4% of all deaths, and alcohol is a causative factor for general illness and injuries. Currently, the alcohol situation in Ukraine is quite devastating. [12, 13]. Chronic ethanol intoxication is manifested by a wide range of effects of various negative factors on the body. Currently, alcohol remains one of the most common toxic factors in everyday life. [14].

Objectification of the findings of the study is achieved by the morphometric method, which enables detection

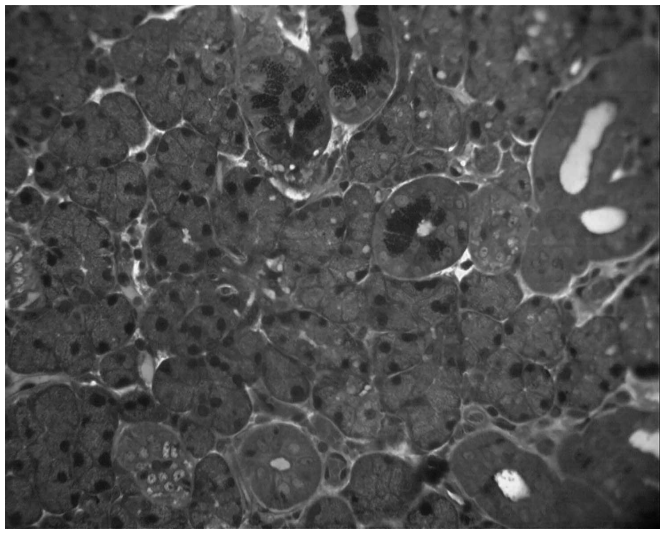

Fig. 1. Intercalated duct of the parenchyma of the lobule of the rat submandibular gland on day 9 of the experiment. Semi-thin section. Methylene blue stain. 400×magnification.

of changes in the structural elements of organs after the effect of various endogenous and exogenous factors [15].

THE AIM

The paper was aimed at the determination of the dynamics of changes of metric parameters of the duct system of the rat submandibular glands in normal condition and chronic ethanol intoxication.

MATERIALS AND METHODS

50 albino outbred rats were involved in the experiment. 10 animals were assigned in the control group and were administered with isotonic saline solution QID, delivered directly into the stomach. 40 animals were assigned in the experimental group, who were administered with 12 mg/kg ethanol 40° QID, delivered directly into the stomach [16]. Animals were withdrawn from the experiment on day 5, 9, 12 and 30 by thiopental anesthesia overdose (25 mg / kg). Pieces of the submandibular glands were embedded into epon-812 according to the conventional technique [17]. Semi-thin sections were stained with methylene blue. The mean values of the outer diameter, the diameter of the lumen of the ducts and the height of the epithelial cells were determined using a Biorex-3 BM-500T microscope with a digital microphotohead DCM with software, adapted to these studies. Statistical processing of morphometric data was performed using the Exel program [18]. Animal housing and experiments on them have been carried out in compliance with the "General Ethic Rules for Conducting Experiments on Animals", adopted by the I National Congress on Bioethics and the requirements of international principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [19].

RESULTS AND DISCUSSION

Morphometric study of the intercalated ducts of the submandibular glands of rats of control group has established that their outer diameter was 18,68±1,07 μm and the lumen diameter was 3,28±0,02 μm. The height of the epithelial cells was 7,08±0,07 μm (Table I).

On day 5 of the experiment the outer diameter of the intercalated ducts reduced by 9,04 % compared to the controls ($p< 0,05$). The lumen diameter reduced by 9,15 %. The height of the epithelial cells was 7,06±0,04 μm, showing no significant difference from the parameters in the controls ($p< 0,05$) (Table I).

On day 9 of the experiment it was established that the value of the outer diameter of the intercalated ducts of the rat submandibular glands no significant difference from the findings of the previous time period of the experiment ($p< 0,05$); however, it was by 11,64 % less than the values in the controls. The lumen diameter of the intercalated ducts reduced by 1,34 % compared with the day 5 of the experiment, though was significantly less by 10,37 % compared with the values in controls ($p< 0,05$). The height of the epithelial cells reduced by 4,96 % compared with the day 5 of the experiment that was significantly less by 5,22 % compared with the values in controls ($p< 0,05$). Its mean values were 6,71±0,06 μm (Table I).

The wall of the intercalated duct was formed by the cuboidal cells with basophilic cytoplasm and eccentric nucleus; the lumen of the duct was narrowed (Fig. 1).

On day 12 the outer diameter of the intercalated ducts was by 12,74 % significantly less than the values in controls ($p< 0,05$). The lumen diameter enlarged by 3,74 % compared with the values of day 9; however, it was by 7,01 % less than the value in controls ($p< 0,05$). The height of the epithelial cells reduced by 1,19% compared with the values of day 9 of the experiment and was by 6,63% significantly less than the values in control rats ($p< 0,05$) (Table I).

Table I. Morphometric parameters of the intercalated ducts of the submandibular glands (μm)

Intercalated ducts	Outer diameter	Lumen diameter	Height of the epithelial cells
Control group	18,68±1,07	3,28±0,02	7,08±0,07
Day 5	16,99±1,05 *	2,98±0,02 *	7,06±0,04
Day 9	16,58±1,03 *	2,94±0,01 *,**	6,71±0,06 *,**
Day 12	16,30±1,03 *	3,05±0,01 *,**	6,63±0,04 *,**
Day 30	16,18±1,04 *	3,06±0,01 *	6,48±0,05 *,**

Note: * - $p < 0,05$ compared to the controls; ** - $p <0,05$ compared with the previous time period of observation.

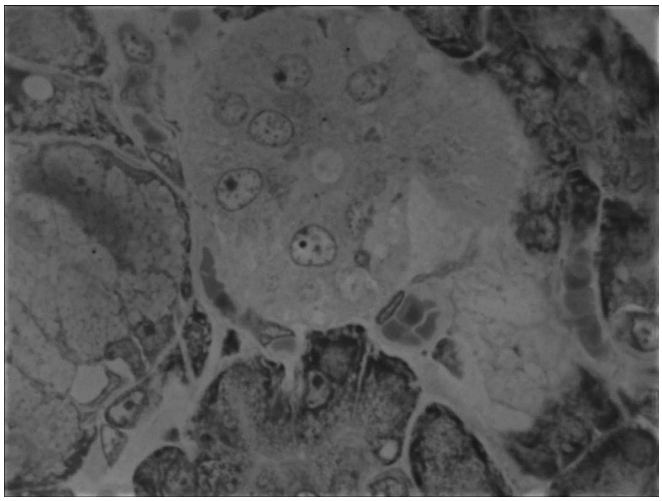

Fig. 2. Striated duct of the lobules of the rat submandibular gland on day 12 of the experiment. Methylene blue stain. 1000×magnification.

On day 30 of the experiment the outer diameter of the intercalated ducts of the submandibular glands differed insignificantly from the value of the previous time period of the experiment and was by 13,38 % significantly less than the values in control animals (p< 0,05). Similarly, the lumen diameter differed insignificantly from the value on day 12 of the experiment, though was by 6,71 % significantly less than the findings in controls (p< 0,05). The height of the epithelial cells was by 2,26 % less than the values in the previous time period of the experiment and by 8,47% significantly less than the values in controls (p< 0,05) (Table I).

The morphometry of the striated ducts of the submandibular glands of rats of control group has established that their outer diameter was 34,80±2,05 μm and the lumen diameter was 5,10±0,03 μm. The height of the epithelial cells was 14,43±1,07 μm (Table II).

On day 5 of the observation the outer diameter of the striated ducts enlarged insignificantly. The mean value of the lumen diameter was by 5,29 % significantly greater than the values in controls. The height of the epithelial cells was almost similar to the values in control rats (p< 0,05) (Table II).

On day 9 of the experiment the values of the outer diameter of the striated ducts of the submandibular glands did not differ from the values of the previous time period of the experiment and its values in controls (p< 0,05). The lumen diameter changed insignificantly compared with the values of the previous time period of the experiment; however, it was by 6,67 % significantly greater than the values in controls (p< 0,05). The height of the epithelial cells was by 10,26 % significantly greater than the values in control animals (p< 0,05) (Table II).

On day 12 of the experiment the outer diameter of the striated ducts of the submandibular glands reduced by 11,54 % compared with the previous findings of the experiment and differed insignificantly from the values in control rats (p< 0,05). The lumen diameter enlarged by 2,02 % compared with day 9 and was by 8,82 % significantly greater than its value in controls (p <0,05). The height of the epithelial cells of the striated ducts of the submandibular glands was by 12,88 % significantly less than the findings of day 9 of the experiment with no significant difference from the values in controls (p <0,05). (Table II).

The wall of the striated duct was formed by the clear epithelial cells. The nuclei were centric. Vacuoles were not numerous. The cytoplasm was homogenous. The basal striation was poorly identified (Fig. 2).

On day 30 of the experiment the outer diameter of the striated ducts was with no significant difference from the values of the previous time period of the observation and values in controls (p <0,05). The lumen diameter enlarged by 2,16 % compared with day 12 of the experiment and was by 11,18 % significantly greater than the values in controls (p<0,05). The height of the epithelial cells of the striated ducts of the submandibular glands was by 8,66 % significantly less than the values of the previous time period and by 12,27 % less than the values in control animals (p <0,05) (Table II).

The wall of the striated ducts was formed by the highly prismatic cells. The lumen of the ducts of rat submandibular glands was filled with optically clear secret. In the basal segments of the epithelial cells of the striated ducts vacuole-like extensions were visualized that become larger in size during the experiment (Fig. 3).

Morphometric study has established that the mean values of the outer diameter of the granular ducts of rats of control group were 38,38±0,05 μm, the lumen diameter was 8,56±0,06 μm, and the height of the epithelial cells was 15,47±0,43 μm (Table III).

On day 5 of the experiment the outer diameter of the granular ducts of the submandibular glands differed insignificantly from the values in control rats, whereas the lumen diameter reduced by 2,45 %. The height of the epithelial cells was by 12,61 % greater than the values in control group of animals (p <0,05). On day 9 of the experiment

Table II. Morphometric parameters of the striated ducts of the submandibular glands (μm)

Striated ducts	Outer diameter	Lumen diameter	Height of the epithelial cells
Control group	34,80±2,05	5,10±0,03	14,43±1,07
Day 5	37,12±2,11	5,37±0,04 *	15,76±1,06
Day 9	37,61±1,94	5,44±0,05 *	15,91±1,09 *
Day 12	33,27±1,81 **	5,55±0,05 *,**	13,86±1,09 **
Day 30	33,48±1,72	5,67±0,03 *,**	12,66±1,09 *,**

Note: * - p < 0,05 compared to the controls; ** - p <0,05 compared with the previous time period of observation.

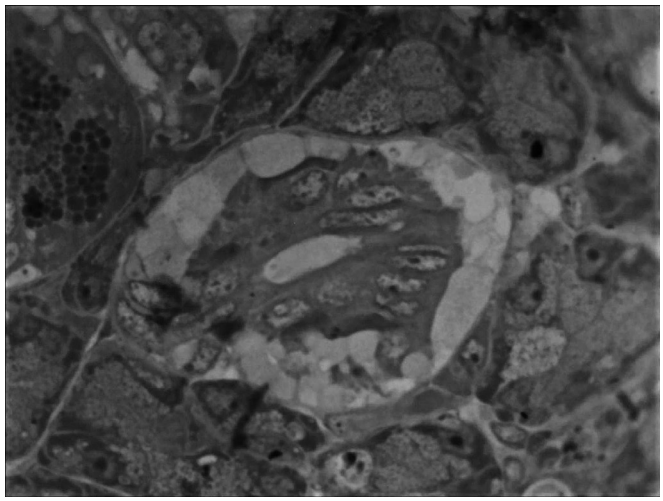

Fig. 3. Vacuoles in the basal part of the epithelial cells of the striated duct on day 30 of the experiment. Semi-thin section. Methylene blue stain. 1000×magnification.

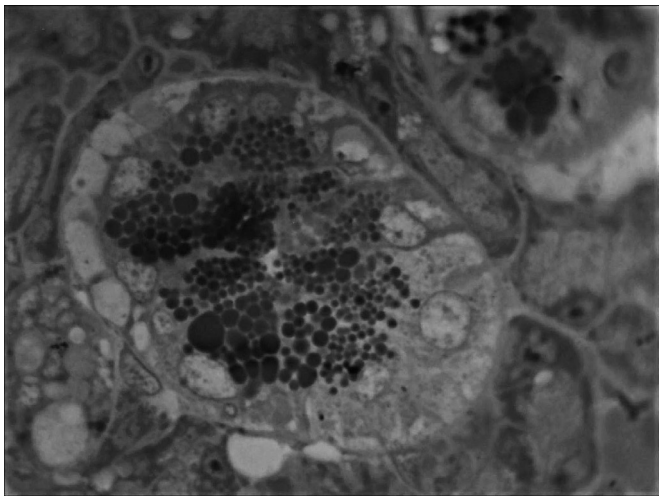

Fig. 4. Granules in the epithelial cells of the granular ducts in the lobules of the rat submandibular glands on day 12 of the experiment. Semi-thin section. Methylene blue stain. 1000×magnification.

Table III. Morphometric parameters of the granular ducts of the submandibular glands (μm)

Granular ducts	Outer diameter	Lumen diameter	Height of the epithelial cells
Control group	38,38±0,05	8,56±0,06	15,47±0,43
Day 5	39,31±0,10	8,35±0,08 *	17,42±0,36 *
Day 9	41,19±0,17 *,**	9,07±0,09 *,**	17,91±0,54 *
Day 12	45,23±0,17 *,**	9,83±0,08 *,**	18,52±0,46 *,**
Day 30	33,58±0,05 *,**	9,36±0,07 *,**	13,62±0,35 *,**

Note: * - p < 0,05 compared to the controls; ** - p <0,05 compared with the previous time period of observation.

the mean value of the outer diameter of the granular ducts of the rat submandibular glands was by 4,78 % greater than the values on day 5 and by 7,32 % greater than the values in controls (p <0,05). The lumen diameter was by 8,62 % greater than the values of the previous time period of the experiment and by 5,96 % greater than the findings in the control group of animals. The height of the epithelial cells was differed insignificantly from the values of the previous time period of the experiment; however it was by 15,03 % significantly greater compared with findings in controls (p <0,05). On day 12 of the experiment the outer diameter was by 9,81 % significantly greater than the values on day 9 and by 17,85 % greater than the values in controls (p <0,05). The mean value of the lumen diameter was by 8,38% greater than the values of the previous time period of the experiment and by 14,84 % greater than the values in control group of animals (p <0,05). The height of the epithelial cells was by 3,41 % greater than the value on day 9 of the experiment and by 19,72 % significantly greater than the findings in control rats (p <0,05) (Table III).

The wall was formed by the single layer of the secretory columnar epithelial cells of variable size and amount that contained optically dense basophilic granules in the cytoplasm. In the basal segments of the epithelial cells of the granular ducts the vacuole-like extensions were visualized. The eccentric nuclei were located in the basal segment of the epithelial cells (Fig. 4).

On day 30 of the experiment the mean value of the outer diameter of the granular ducts of the rat submandibular glands was by 25,76 % significantly less than the values on day 12 and by 12,51 % less than the value in controls (p <0,05). The lumen diameter also reduced by 4,78 % compared to day 12 and enlarged by 9,35 % compared with controls (p <0,05). The height of the epithelial cells of the granular ducts of the submandibular glands was by 26,46 % less than the value in the previous time period of the experiment and by 11,96 % less than the findings in controls (p <0,05) (Table III).

The previous experiments showed multidirectional response of the duct system on the state of salivary glands under the influence of various exogenous factors. In that case, on day 14 of the experiment under the effect of methacrylate the mean values of the outer diameter and lumen diameter of the intralobular ducts decreased, tending to reduce in size to the end of the experiment, which was the result of hyperhydration of the amorphous substance due to microcirculation disorder [20], in contrast to the effect of ethanol, when similar changes occurred in the intercalated ducts, which also responded by persistent narrowing with reduce in the height of the epithelial cells due to microcirculation disorder in the exchange section, which is confirmed by the reduced outer diameter by 19.5%, lumen diameter by 12.5% and thinning of the vascular wall by 30.4%, [21], which resulted in hyposalivation, since intercalated ducts regulate the quantitative aspect of salivation.

Noteworthy, the mean values of the outer and inner diameters of the striated and granular ducts increased with the subsequent regeneration, due to the compensatory reaction to the narrowing of the intercalated ones to increase the amount of saliva through the juxtacellular transport of liquid from the surrounding interstitium on the side of the striates ducts [9] and the provision of system of local mechanisms for regulating blood flow to the vascular system due to granular ducts with kallikrein granules, leading to depletion of the epithelial cells of the duct system of the submandibular salivary glands at the end of the experiment. It was confirmed experimentally by a decrease in the mean values of the height of the epithelial cells compared to the values in control group of rats, though tending to recovery since ethanol is a metabolite in contrast to methacrylate, which acts as a toxic substance, and the location of the submandibular glands outside the oral cavity, affected indirectly through the vessels of the microvasculature.

CONCLUSIONS

To conclude with, the duct system responds to the effect of chronic ethanol intoxication, which at the initial stage of the experiment is confirmed by the narrowing of the outer and inner diameters with reduced height of the epithelial cells of the intercalated ducts, enlargement of the outer and inner diameter with increased height of the epithelial cells of the striated and granular ducts with a tendency to regeneration in the second half of the experiment. However, no recovery of the parameters by day 30 of the experiment was registered, which obviously indicates the depletion of the secretory epithelium of the duct system, due to dystrophic changes caused by vascular disorder in the microvasculature, confirmed by changes in the diameters of the walls of the ducts with a decrease in the height of epithelial cells.

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The Authors declare no conflict of interest

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ORIGINAL ARTICLE
PRACA ORYGINALNA

APPLICATION OF COGNITIVE-BEHAVIORAL THERAPY IN PATIENTS WITH UNCONTROLLED BRONCHIAL ASTHMA DUE TO EXCESS BODY WEIGHT AND OBESITY

DOI: 10.36740/WLek202001126

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ABSTRACT

The aim: The aim of our study was to determine the effect of CBT in patients with uncontrolled BA against the background of excess body weight and obesity.
Materials and methods: By the study design there were examined 78 patients who had a primary diagnosis of uncontrolled BA.
Results: The patients in the main group had significant positive dynamics regarding a number of AQLQ questionnaires, namely emotions, activity, overall quality of life ($p \leq 0.05$). These patients also had a positive dynamics in overcoming anxiety-depressive disorders, namely from 10.23 ± 0.81 points anxiety disorders scored 7.65 ± 0.98 points after CBT, and depressive changes – from 10.01 ± 0.79 points to 7.69 ± 0.67 points by the HADS scale ($p \leq 0.05$).
Conclusions: The use of CBT has a positive effect on certain indices of the AQLQ questionnaire ($p \leq 0.05$) and has a positive effect on the psychological state of the patient, namely, it reduces the manifestations of anxiety and depression ($p \leq 0.05$) in patients with uncontrolled asthma as well as improves the course of uncontrolled asthma by a number of indices, namely reducing the frequency of use of short-acting β-2-agonists, nocturnal symptoms of asthma, the amount of oral and peroral steroids.

KEY WORDS: bronchial asthma, cognitive – behavioral therapy (CBT), EBW, anxiety-depressive disorders (ADD)

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INTRODUCTION

Bronchial asthma (BA) is a chronic respiratory disease characterized by the manifestations of such symptoms as wheezing, shortness of breath, chest tightness, which vary greatly over time in different people [1]. Recent studies have suggested that more than 334 million people on the Earth suffer from asthma, and it has been found that the cost of asthma is one of the largest in the world among non-communicable diseases [2].

At the same time, there are a number of diseases that have a similar pattern, such as shortness of breath, chest tightness, coughing, palpitations, inability to complete the phrase, etc. In our opinion, the most common among them in the adult population are anxiety (AD) and depressive (DD) disorders, which may have a character a comorbid disease in patients with asthma, potentiating more severe and less controlled asthma [3,4].

According to a number of studies, patients with asthma have a higher rate of AD and DD than in the population [5]. It is established that depending on the severity of the course 22-45% of patients have comorbid pathology in the form of BA against the background of DD, and AD and / or from 6.5 to 26% of patients have panic disorder (PD) [6,7,8]. According to GINA 2016, the presence of psychological disorders in patients with asthma is associated with a more severe asthma and more visits to the hospital, especially for people from deprived socio-economic and ethnic backgrounds [2].

Another pressing issue for patients with asthma is the presence of excess body weight (EBW) or obesity, which is also an aggravating factor in controlling the disease. Patients with EBW or obesity have been found to have a low disease control and poor response to traditional therapy [9,10,11].

Patients with insufficient control and comorbid diseases, mentioned above, have been found to use fast-acting bronchodilator (FABD) more often than patients with BA alone [12]. The use of FABD has many side effects, reduces the response to basic therapy and, even leads to an increase in lethal events among the population of patients with BA. At the same time, it has been established that the presence of patients with BA with AD or DD leads to poor keeping basic therapy and such patients more often do not keep the recommended lifestyle (for example: quitting smoking, avoiding contact with allergens, etc.) [13,14].

Recent studies indicate a positive effect of cognitive-behavioral therapy in the treatment of patients with asthma [15].

Cognitive – behavioral therapy (CBT) is a complex of psychotherapeutic measures that includes behavioral and cognitive therapies. The cognitive approach is based on the assumption that psychological problems and neuro-psychiatric disorders are recognized as illogical inappropriate thoughts and beliefs of a person, as well as dysfunctional stereotypes of his thinking, which can be solved if changed. The behavioral approach, based on the theory of behaviorism, involves changes in the human behavior by encouraging and reinforcing desirable behavior forms and absence of re-

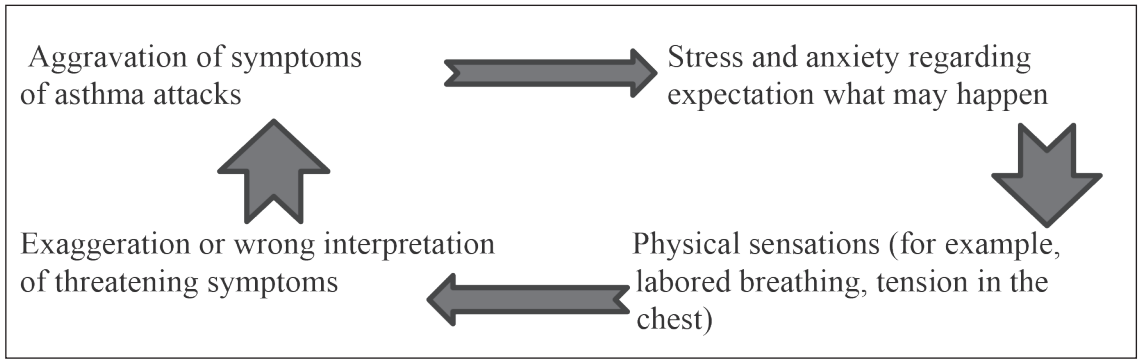

Fig 1. The vicious circle of patients' concerns as to asthma

inforcement of undesirable forms. In our case, cognitive-behavioral therapy is a treatment-and-preventive measure that is a cognitive tool of the patient's self-influence [16].

In the context of chronic diseases, and BA is precisely such a disease, a person is confronted with certain aspects of the disease that can be troublesome or difficult to cope with by himself, which in turn leads to physiological reactions that worsen the course of the underlying disease. CBT aims at breaking the vicious circle of patients with asthma (Fig. 1).

Concerning the relevance of CBT in patients with EBW or obesity, we have the following data: CTC has traditionally been considered as one of the best treatments for EBW or obesity [17]. In some publications, this method is considered as the first-line treatment among psychological approaches [18]. Although data suggest positive results, some studies indicate that this psychotherapy does not necessarily lead to successful loss of excess body weight [19] in comparison with placebo [20,21].

In his study DalleGrave with co-authors described a typical CBT protocol, which reported a set of procedures needed to eliminate obstacles to losing and maintaining a patient's body weight [22].

As early as 2004, the National Institute of Clinical Excellence recommended to use CBT in the treatment of patients with EBW or obesity with an A degree of evidentiality.

Taking into account all of the above, there is a need to find additional methods in the treatment of patients with asthma against the background of EBW or obesity, as we believe, this may be alongside with basic cognitive-behavioral therapy, namely in patients with uncontrolled overweight and obesity.

THE AIM

The aim of our study was to determine the effect of using cognitive-behavioral therapy in patients with uncontrolled course of asthma in presence of EBW and obesity.

MATERIALS AND METHODS

The study was conducted on the basis of the Department of Family Medicine and General Practice. According to he study design there were examined 78 patients who had a primary diagnosis of uncontrolled BA. The study involved adults from 19 to 57 years of age. The patients were divided into groups, namely the main group included 39 (50.00%) patients who were offered CBT and 39 patients (50.00%) who refused CBT and formed a comparison group that received only basic treatment.

In the development of CBT, we were guided by certain principles that aided in the work with the patient, namely: CBT is based on the formulation of the patient's problem and the individual conceptualization of each patient within a cognitive approach; CBT involves creating a strong therapeutic alliance between the physician and the patient; special attention in CBT is given to the cooperation and active involvement of the physician in assisting the patient; CBT is aimed at the result and focused on issues that are fundamental to the clinical course of the underlying disease; first of all, CBT emphasizes the current concern of the patient today; CBT is first and foremost an educational therapy, its purpose is to teach the patient to be a doctor for himself; CBT should be time-limited, in our case it was 15 sessions; CBT sessions should be structured; CBT teaches patients to identify, evaluate, and respond to their dysfunctional thoughts and beliefs; CBT uses a number of techniques to help change mode of thoughts, mood and behavior.

CBT developed by us included, as indicated above, 15 sessions: the first six sessions were twice a week, then six sessions were once a week, the last 3 sessions were once every two weeks. That is only 15 weeks of therapy. Then, at the request and need of the patient, sessions were offered once every three months during the year.

The session was structured and had the following parts: introductory part (mood check, short discussion of the last week, collaborative formation of the session plan), middle part (discussion of homework, current problems according to the plan, new homework definition, summarizing), and concluding part (receiving feedback).

To establish positive dynamics in the course of the underlying disease, namely BA, the following criteria were offered to our patients: quality of life assessment (AQLQ (quality of life in asthma)), symptom control (the number of FABD used per week, the amount of nocturnal symptoms, response to the baseline therapy, asthma control (AST test), use of oral and peroral steroids), changes in AD and DD were diagnosed using the Hospital Alarm and Depression Scale (HADS). These diagnostic criteria were applied in the first, eighth and fifteenth sessions.

Statistical analysis was carried out by conventional methods of variational statistics. Reliability was assessed by Student's t test. The differences were considered reliable at the significance level of $p \leq 0.05$. Correlation was established

Table I. AQLQ quality of life assessment at the first visit in patients with uncontrolled bronchial asthma

Scale	Groups	
	Main group	Comparison group
Activity	3.7±0.1	3.6±0.2
Symptoms	3.5±0,3	3.5±0,1
Emotions	3.6±0.1	3.5±0.5
Environment	3.4±0.2	3.4±0.3
General life quality	3.5±0.1	3.5±0.2

Note: * p<0.05

Table II. The disease course and symptom control at the first visit in patients with uncontrolled bronchial asthma

Criterium	Group	
	Main	Comparison
Use of FABD per week	8.7±1.2	8.5±0.8
Response to basic therapy	Unsatisfactory	Unsatisfactory
AST-test	11.8±1.5	12.1±1.9
Use of oral steroids per week	4.3±0.8	4.5±0.9
Use of peroral steroids per week	2.1±0.9	2.2±1.1
Night symptoms per week	4.3±0.5	4.2±0.9

Note:* p<0.05

Table III. AQLQ Quality of Life Assessment in Dynamics of observation

Scale	Groups			
	Main group		Comparison group	
	1st visit	15th visit	1st visit	15th visit
Activity	3.7±0.1*	5.8±0.2	3.6±0.2	3.6±0.3
Symptoms	3.5±0.3	5.2±0.8	3.5±0.1	3.6±0.2
Emotions	3.6±0.1*	5.4±0.4	3.5±0.5	3.4±0.2
Environment	3.4±0.2	4.9±0.8	3.4±0.3	3.5±0.1
General life quality	3.5±0.1*	5.7±0.2	3.5±0.2	3. 5±0. 1

Note: * p<0.05

using Spearman and Pearson correlation coefficients.

The study was conducted with the written consent of the patient and with the requirements of bioethics.

RESULTS AND DISCUSSION

Presently, patients with comorbid pathology in the form of EBW or obesity have been found to have a more uncontrolled course of BA, which has a lower response to the baseline therapy.

Our study confirms this hypothesis, so it was found that the majority of the patients in the main group and the comparison group who had uncontrolled asthma had EBW or obesity by the design of the study, namely 41.03% of patients in both groups, and 32 patients had comorbid pathology as obesity and 41.03% of patients had EBW.

At the same time, it should be noted that a number of studies indicate gender differences in the incidence of obesity or EBW, in our study we also confirm this, so it was found that the main group included 27 women and the comparison group – 25. That is, the majority of patients with uncontrolled asthma against the background of EBW or obesity are women.

During history taking it was found that patients with uncontrolled course of asthma had comorbid pathology not only in the form of EBW or obesity, but also hypertension in 37 patients (47.43%), gastroesophageal disease in 33 patients (42.31%), chronic obstructive disease of the lungs was found in 7 patients (8.97%), and diabetes was diagnosed in 2 patients.

At the first visit, all patients were asked to complete a quality of life questionnaire (AQLQ (BA quality of life)). The data received are presented in the Table I.

Table 1 shows that patients with uncontrolled BA have low quality of life scores and no statistically reliable difference between groups.

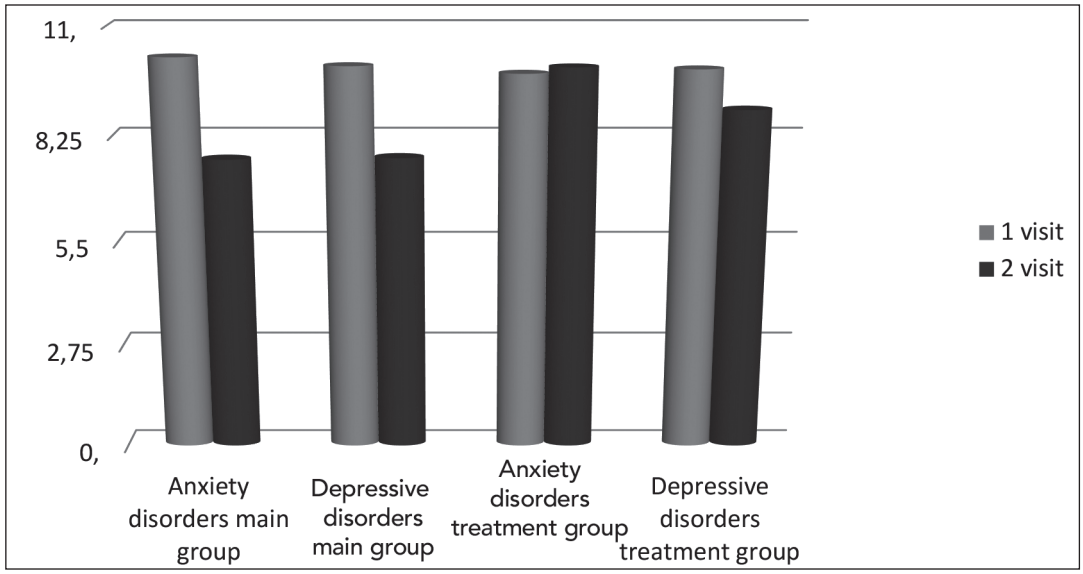

Fig 2. Dynamics of the HADS scale in the patients with uncontrolled asthma against the background of EBW or overweight.

Manifestations of anxiety-depressive disorders (ADD) were detected in 44 (56.41%) patients in the main group. At the same time clinically expressed ADD by the HADS scale in the main group were observed in 35.89% of patients, subclinically expressed in 20.51% (mean score 21.7 ± 1.4 and 9.4 ± 1.2, respectively).

Together with the above questionnaires, the patients also answered questions on the disease course and symptom control. The data are presented in the Table II.

According to the design of the study, the patients in the main group were offered CBT together with baseline therapy, which included 15 sessions, and the patients in the comparison group who refused the proposed therapy received only baseline treatment.

It should be noted that all patients in the main group willingly attended CPT sessions and improved compliance with the attending physician. The patients were motivated to change attitudes toward their underlying disease, namely, asthma, and were strongly encouraged to lose weight in those patients who had comorbid abnormalities such as EBW or obesity.

According to the design of the study, the feasibility of using CBT in the patients with uncontrolled course of asthma against the background of EBW or obesity was based of a number of criteria, the first one was the AQLQ questionnaire (Table III).

Table III shows that the patients in the main group had reliable positive dynamics in a number of indices, namely emotions, activity, overall quality of life ($p \leq 0.05$). Such indices as symptoms and the environment also gained positive dynamics, however, when statistically calculated, they did not have reliable indices ($p \geq 0.05$), whereas the comparison patients receiving only baseline therapy had no significant changes in the quality of life in BA.

The patients of the main group had positive dynamics in overcoming anxiety and depressive disorders. These indices, according to the study design were diagnosed using the HADS scale (Fig. 2)

Fig. 2 shows that patients in the main group had reliable positive dynamics in overcoming ADD, namely from 10.23 ± 0.81 points anxiety disorders acquired 7.65 ± 0.98 points after CBT, and depressive ones changed from 10.01 ± 0.79 points to 7.69 ± 0.67 points by the HADS scale ($p \leq 0.05$). The patients in the treatment group receiving baseline therapy had no reliable changes in ADD by the HADS scale ($p \geq 0.05$).

Cognitive-behavioral therapy also had a positive effect on the course and control of the disease, namely the patients in the main group began to use rescue medications less frequently, and FABD per week reduced from 8.7 ± 1.2 at the first visit to 5.1 ± 0.9 at the 15th visit ($p \leq 0.05$). The number of such threatening predictors as the amount of nocturnal symptoms per week decreased, at the first visit patients noted 4.3 ± 0.5 and there were 3.1 ± 0.3 nocturnal attacks of asthma at the 15th visit ($p \leq 0.05$). The patients who had undergone CPT improved asthma control, namely by the result of the AST test, which was 15.6 ± 1.1 points at the 15th visit versus 11.8 ± 1.5 at the first visit. All of the above changes are related to the primary group, the comparison group patients did not have any change, and even 7 patients experienced worsening of the underlying disease.

As for changes in the treatment of asthma in the patients of the main group and the comparison group, no positive dynamics were recorded regarding the response to the baseline therapy during the period of the follow-up, and it remained unsatisfactory. However, the patients in the main group who underwent CBT in addition to basic treatment reduced the use of oral and peroral steroids 1.5-2 times, whereas the patients in the comparison group did not obtain such results.

CONCLUSIONS

1. Bronchial asthma with comorbid pathology in the form of excess body weight and obesity has a more severe and

uncontrolled course, which complicates the patient's treatment with baseline therapy.
2. Patients with uncontrolled bronchial asthma have a low standard of life quality according to the AQLQ questionnaire, and the majority of patients, namely 56.41%, have anxiety disorders by the HADS scale.
3. The use of cognitive – behavioral therapy has a positive effect on certain indices of the AQLQ questionnaire ($p \leq 0.05$) and has a positive effect on the patient's psychological state, namely, reduces the manifestations of anxiety and depression ($p \leq 0.05$) in patients with uncontrolled asthma.
4. Cognitive-behavioral therapy improves the course of uncontrolled bronchial asthma in a number of indices, namely reducing the frequency of use of short-acting β-2-agonists, nocturnal symptoms of asthma, the amount of oral and peroral steroids used.
5. The additional use of cognitive-behavioral therapy in patients with uncontrolled bronchial asthma against the background of excess body weight or obesity has a positive effect on asthma control according to the results of the AST test ($p \leq 0.05$). Cognitive-behavioral therapy should be obligatory in routine practice for physicians managing patients with bronchial asthma.

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Conflict of interest:
The Author declare no conflict of interest

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ORIGINAL ARTICLE
PRACA ORYGINALNA

ECTOPIC PREGNANCY AND ITS LONG-TERM RESULTS

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ABSTRACT

The aim: The purpose of this study was to assess the long-term outcomes of restoration of reproductive function after surgical and medical treatment of ectopic pregnancy, taking into consideration the patency of the fallopian tubes and the incidence of uterine pregnancy.

Materials and methods: A two-stage experimental approach was used to address research objectives. In the first stage, a retrospective analysis of 615 histories of patients with ectopic pregnancy has been performed. In a second stage – we examined 140 patients, which were divided into three groups, depending on the type of treatment. The first group consisted of patients with a disturbed ectopic pregnancy, who were treated with laparotomy and tubectomy. The second group included patients with exacerbated ectopic pregnancy, who were operated by laparoscopic access. Lastly, the third group comprised of women with ectopic pregnancy who were treated with methotrexate. The main source of information used for clinical and anamnestic analysis was "medical card-patient" (f. 003 / o).

Results: The number of patients diagnosed with ectopic pregnancy increased from 2005 to 2015. In 2005 and 2006, the laparotomy operations were 86.88% and 83.33%, but conservative management only 13,16% and 16,67%, respectively. In 2015, the number of patients treated with methotrexate was more than half (51.35%) compared with 2010 and 2005 increased to 16.97% and 38.19% respectively, and laparotomy operations decreased from 86.88% in year 2005 to 18.92% in year 2015.

Conclusions: The data showed that in women who underwent medical treatment with cytostatic, the patency of the fallopian tubes was significantly better than after surgical treatment. In cases of interrupted ectopic pregnancy for which laparotomy with the removal of the motor tube was applied, infertility of tubal peritoneal genesis developed in 60% of cases, which is consistent with the existing literature. Statistical analysis of the structure of ectopic pregnancy showed that in 2005 dominated interrupted ectopic pregnancy, due to late diagnostic and hospitalisation, that led to urgent laparotomy operations rather than conservative treatment.

KEY WORDS: ectopic pregnancy, methotrexate, infertility, removal of the fallopian tube, metrosalpingography

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INTRODUCTION

Ectopic pregnancy is one of the main causes of reproductive loss in women with the tubal factor being39% ofinfertility structure. The risk forlethality fromectopic pregnanciesis 10 times higher than that of childbirth and 50 times higher than that during an artificial abortion [1, 2]. Particularly relevant is the problem of the first ectopic pregnancy (EP), which results in 50-80% of the patients undergoing a reproductive function after its surgical treatment, and the frequency of repeated ectopic nidations is 7 -17% [3, 4].

According to the literature, the conservative treatment of progressing EP by methotrexate under the control of of chorionic gonadotrophin levels leads to complete degeneration of the fetal egg [5, 6].Operative treatment of EP by laparoscopic access in comparison with laparotomy has a number of significant advantages, such as: small invasiveness, narcotic analgesicsrefusal, faster physical, sexual and social rehabilitation, cosmetic effect, reduction of purulent-septic complications. Indications and contraindications to the operative and conservative management of progressive ectopic pregnancy remain still current [7, 8].

Given the evidence presented, it is necessary to develop an advanced algorithm for progressive ectopic pregnancy and a method of operative technique that will provide an opportunity for optimal endosurgical intervention in the case of interrupted ectopic pregnancy. Therefore, the purpose of thisstudywas to elucidatethe long-term outcomesof restoration of reproductive function after surgical and medical treatment of ectopic pregnancy, according to the patency of the fallopian tubes and the incidence of uterine pregnancy.

THE AIM

The purpose of this study was to assess the long-term outcomes of restoration of reproductive function after surgical and medical treatment of ectopic pregnancy, taking into consideration the patency of the fallopian tubes and the incidence of uterine pregnancy.

MATERIALS AND METODS

This research was conducted inthe gynecological department of Ternopil municipal communal hospital №2 affiliated with the Department of Obstetrics and Gynecology of I.Horbachevsky Ternopil National Medical University. The study was conducted in accordance with ethical principles of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and the relevant laws of Ukraine on conducting experimental and clinical

Table 1. Research design

Groups of patients	subgroups	Methods of treatment
I (n=20)		tubectomy viaa laparotomy access
II (n=40)	IIA (n=20)	removal of fertilized egg by laparoscopic access with preservation of the tube
	IIB (n=20)	tubecotomy vialaparoscopic access
III (n=80)	III A (n=20)	methotrexate
	IIIB (n=20)	methotrexate followed by removal of fetal egg vialaparoscopic access with preservation of the tube
	IIIC (n=20)	methotrexate followed by laparoscopic tubectomy
	IIID (n=20)	methotrexate followed by tubectomy via a laparotomy access

studies. The patients gave informed consent to participate in the study. The protocol of the study was approved by the Commission on Bioethics of I.Horbachevsky Ternopil National Medical University.

The source of information used for clinical and anamnestic analysis was «Patients' medical records» (p. 003 / o) of the patients undergoing ectopic pregnancy. Ectopic pregnancy was diagnosed as criteria for inclusion of patients in the study. The groups were selected in accordance with the aim and objectives of this study. The control group consisted of 30 women with the physiological course of pregnancy in the first trimester.At the second stage of the study, we examined 140 patients, which were divided into threegroups, depending on the type of treatment (Table 1).

The first group consisted of patients with a disturbed ectopic pregnancy who were treated with laparotomy and tubectomy. The second group included patients with exacerbated ectopic pregnancy, who were operated by laparoscopic access, and the third group – comprised of women with EP, who were treated with methotrexate in a dose of 75-100 mg intramuscularly. If there was no effect of drug treatment, ectopic pregnancy continued to develop, patients were given prompt treatment. Depending on the type of treatment, groups were randomly divided into subgroups (Table 1) in order to adequately assess the results of the surveys.

The inclusion criteria for using methotrexate were as follows: the diameter of the fetal egg not more than 3.5 cm in the area of uterine application (according to ultrasound data), and the level of β-HCG of no more than 1500 IU / l as determined by the ECLIA analyzer Cobas 6000 (Roche Diagnostics, Rotkreuz, Switzerland).

The laparoscopic operation was performed using instrumentsmade by "KarlStorz" firm (Germany). Theultrasound examination for the diagnosis of ectopic pregnancy was carried out transabdominally and transvaginally usingthe ultrasound device "Aloka SSD-1800" (Toshiba, Japan) with sensors from 3.5 to 10 MHz. Metrosalpingography (MSG) was performed in the radiological department and the department of vascular surgery of Ternopil communal city hospital №2 using the Siemens Axiom Artis 20 dBA_sm15724 angiograph with a digital flat-panel detector.Monitoring of the onset of pregnancy in the examined women was carried out within 6-24 months after treatment.

Statistical analysisof the datawas performed on a personal computer usingthe STATISTICA-10 software for Windows®-6.0 package. Odds Ratio (OR) and its 95% confidence interval (95% Confidential Interval, 95% CI) were used to identify risk factors and predict complications. The reliability of the abolition of a pair of averages was calculated using the Student and Fisher test criteria.

RESULTS AND DISCUSSION

We conducted an analysis of various methods of ectopic pregnancy treatment during 2005 -2015 under conditions of one gynecological hospital. In 2015 the absolute number of ectopic pregnancies exceeded the indicators by 49.4%in 2005, and compared to 2010 – by 28.9% (Fig. 1).

There were significant changes inthe structure of treatment methods from 2005 to 2015, as shown in Fig 2.

In years 2005 and 2006, the share of laparotomy operations was 86.88% and 83.33%, while medical treatment was only 13.16% and 16.67%, respectively (Fig. 2). In 2015, the number of patients treated with methotrexate was more than a half or 51.35%, which in comparison with years 2010 and 2005 increased by 16.97% and 38.19% respectively, whereas laparotomy operations decreased by 86.88% in 2005 to 18.92% in 2015; laparoscopic access was dominantover laparotomic access in 2015, almost twice compared to year 2005 (29.73% and 18.92%, respectively).

Furthermore, it was found that EP is most common atthe age of 26-30. The average age of patients was (25.29 ± 1.75) years (Fig. 3), and in the control group – (23.5 ± 0.62) years. EPdiagnosis was established on the basis of clinical, ultrasound and laboratory methods in 65% of the surveyed patients during the period of 3-4 weeks of gestation, 20% – in 5-6 weeksof gestation, and 15% – in the term more than 6 weeksof gestation. The diagnosis of ectopic pregnancy was established by a transabdominal ultrasound sensor in 40.4% of women, and by transvaginal sensor – in 74.1% of women.

Determination of serum β-HCG level was performed in all patients with progressive ectopic pregnancy (EP).

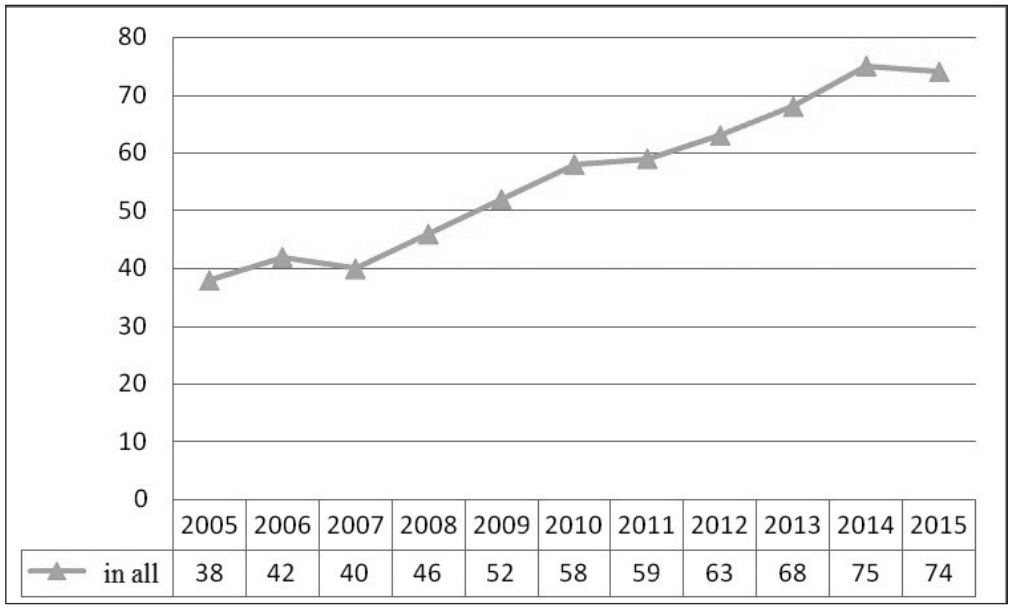

Fig. 1. Number of women with ectopic pregnancy hospitalized in the gynecological department of Ternopil municipal communal hospital №2

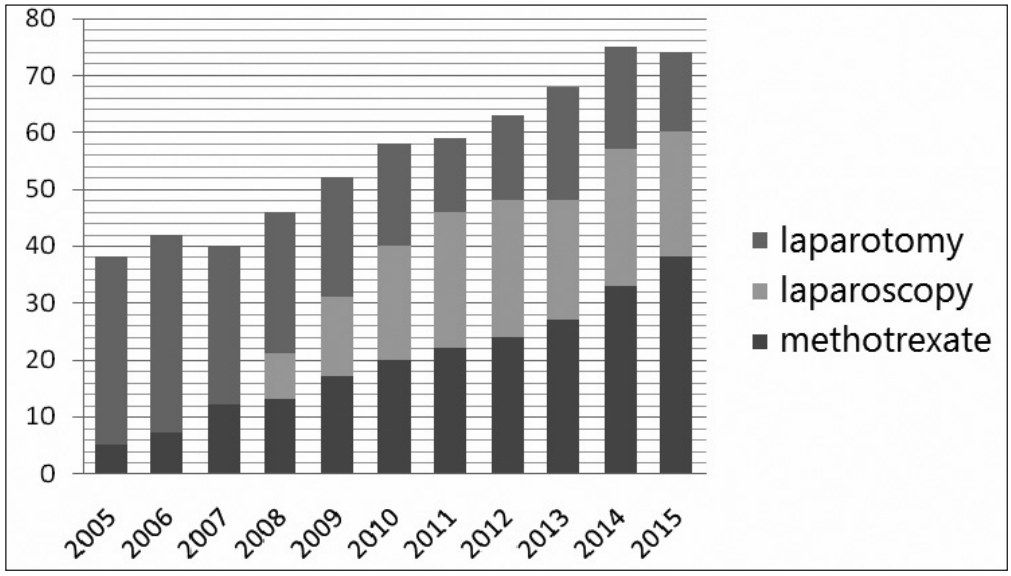

Fig. 2. The number of patients with ectopic pregnancy who were treated with operative (laparotomy, laparoscopic) and medication method.

At 3-4 weeks of pregnancy, the concentrationof β-HCG hormone was 476.25 ± 11.86 IU / L, and 2736.14 ± 142.67 IU / L in patients with ectopic pregnancy in the period of 5-6 weeks, that did not correspond to the reference value of β-HCG hormone during the physiological course of uterine pregnancy in the appropriate period.

For the purpose of differential diagnosis of ectopic pregnancy and uterine pregnancy, the growth of β-HCG was studied in the early stages. Aftera 48-hour re-analysis, the increase in β-HCG levels was less than 1.6-fold compared to the physiological course of uterine pregnancy; a lack of growth or drop of β-HCG testified in favor of ectopic pregnancy.

Patient complaints upon arrival to the clinic were as follows: delayed menstruation – 100%, pain in the lower abdomen 98.68%, vertigo – 48.35%, loss of consciousness – 31.29%, general weakness – 78.40%, and nausea – 8.30% .

The analysis of gynecological history revealed thatmenstrual cycle was regular in 84.45% of women. In 78.93% of women, the cycle lasted from 21 to 28 days, it was more than 29 days in 15.76%, and – 20 or less days in 5.31% of women, as presentedin Fig. 2. The duration of menstruation was on average 3-7 daysin 69.40% of women, it was up to 3 daysin 25.89% of women, and – over 1 week in 4.71% of women (Fig.4).

Menstrual dysfunction in women with ectopic pregnancy was found in 53.00% of patients and was manifested mainly by algmenorrhea in 24% of women, dysmenorrhea in 18.75%, and hypertension in 9.37%, which was 7.3 times that of control group (OR = 7.3; 95% CI: (2.51-21.25); p <0.05) (Fig. 5).

Among the risk factors found during the analysisof anamnestic data of patients, it was found that 40.2% of patients had early puberty (OR = 4.36; 95% CI: (1.50-12.69); p <0.05) , and among different contraceptive methods, intrauterine helix prevailed in 43.4% of cases (OR = 6.9; 95% CI: (2.07-22.00); p <0.05). In 32.76% of women this pregnancy was the first, in 38.31% – the second, in 20.85% – the

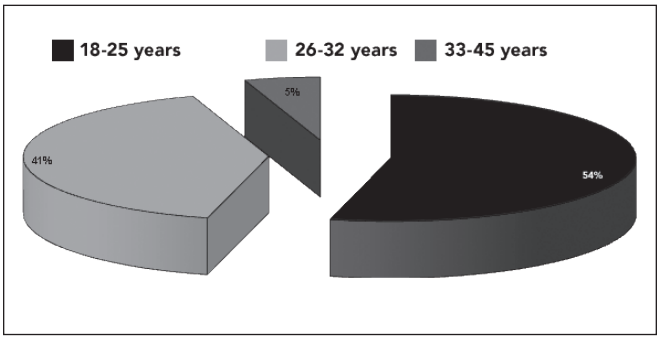

Fig. 3. Distribution of patients by age

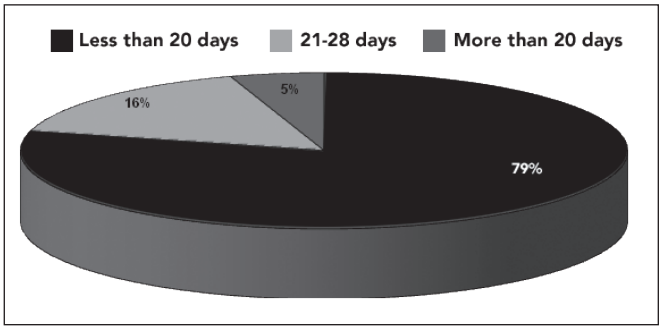

Fig. 4. Duration of the menstrual cycle

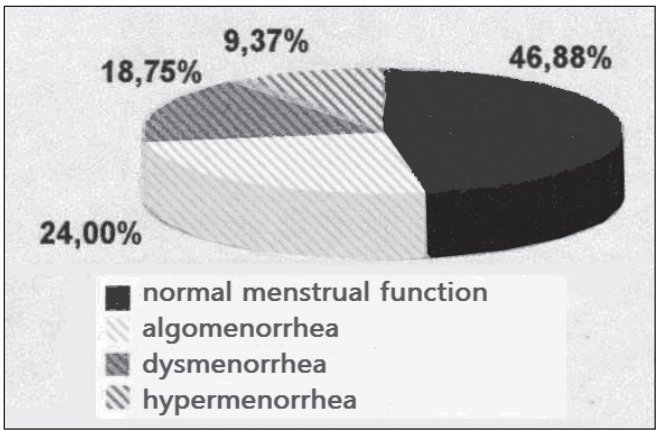

Fig. 5. Characteristics of menstrual function in the surveyed patients

third, in 8.08% – the fourth ormore. 48.74% of women had birth in history. Out of 56.25% total complications from the previous labor and the postpartum period, 28.12% of women had a subinvolution of the uterus, 15.63% – hematometry, and 12.5% – the weakness of labor. Medical abortions wereregistered in 19.2% of patients (OR = 2.13; 95% CI: (0.63-7.16); p <0.05). Among this population, one abortion was reported in 24.4% of women, two – 8.9%, and three of 1.67%. Unauthorized abortions were registeredin 6.5% of the total number of women with ectopic pregnancy.

According to the anamnesis, 24.9% of the surveyed women had bad habits, namely smoking (OR = 2.98; 95% CI: (0.89-9.96); p <0.05). The extragenital diseases were found out in 84.4% of patients with ectopic pregnancy, pediatric infections –in 66.3% (OR = 6.81; 95% CI: (2.05-22.65); p <0.05). Moreover, 65.6% of women with ectopic pregnancy had gynecologic diseasessuch as inflammatory diseases of the uterus and appendages, and benign tumors occurred in 40.7% of patients.

Further analysis of the data derived from 140 patients (the second stage of the study) revealed that in women of group I the restoration of menstruation after surgery occurred in 31,56 ± 2,09 days,in women of group II– in 30.64 ± 1.72 days or less than 3.54%compared togroup I, although it was not statistically significant (p> 0.05). Patients in the third group receiving methotrexate noted a later onset of the next menstrual period (40.78 ± 2.13), which grew by 29.2% (p <0.05) compared with group I, and by group II by 33, 9% (p <0.05).

Table 2 shows the absolute numbers of women with passable, partially passable and obstructed uterine tubes based on the results of MSG. In group II A, where the laparoscopic operation with conservative removal of the fetal egg was performed, the number of women with the passage of the fallopian tubes was 11 or 55%. In group IIIA, where methotrexate was used, this number was 17 (85%), which indicates the best result in 30% of women (p< 0.05).

In group IIIB (patients were administered methotrexate, and then laparoscopic intervention, conservative removal of the fetal egg was performed), women with passable fallopian tubes accounted for 70% (14 patients), which compared togroup IIIA (only methotrexate) was 15% less (p<0.05), but compared with group IIA (nocytostatic) it was better by 15% (p< 0.05). Partially passable tubes were found in 4 patients (20%) ofgroup IIA, 3 (15%) patients of group IIIA, and 4 (20%) patients of group IIIB, however the difference between these indicators were statistically significant (p>0.05). Moreover, women with obstructed uterine tubeswere – 3 (15%) in group IIA and 2 (10%) in group IIB, whereas the fallopian tubes obstruction was not detectedin women of group IIIA.

Patency was evaluated in women who have undergone tubectomy and hada single uterine tube that was not operated. In group I, the passable tube was in 4 women (20%), whereas in group IIB, when performing tubectomy with laparoscopic access, the patency of the single fallopian tube was diagnosed in 12 women (60%), which increased by 40% compared to the laparotomic method (p<0.05).Following the use of methotrexate and laparoscopic tubectomy (groupIIIB), the patency of the fallopian tubes was found in13 (65%) of women, and in 7 (35%)of group III C, which is less in comparison with group III B by 30% (p<0.05). The patency of fallopian tubes after laparotomy tubectomy (groupI) was 20%, which is 15% less (p<0,05) than in the group IIIC with the previous use of cytostatics (35%). In the case of laparoscopic access without using cytostatics (group II B), 12 (60%) of the passable fallopian tubes, and after using methotrexate (group III C) – 13 (65%), (p>0,05). Partially passable fallopian tubes were observed in 35% of womenin groupsI and III B, however it was independent of the use of methotrexate. In the group II B, the fallopian tube, that was not used, preserved partially passable in 4 women (20%), and it was in 6 patients (30%) in group IIIC.

There were 9 (45%) women who were subsequently diagnosed with a tubal peritoneal infertility based on MSG after

Table 2. Fallopian tubes passages in women with an ectopic pregnancy in ana mnesis based on the results of digital and analogue metrosealngography

	Group I n=20	Group II n=40		Group III n=80			
		II A n=20	II B n=20	III A n=20	III B n=20	III C n=20	III D n=20
Passable	4 (20%)	11 (55%)	12 (60%)	17 (85%)*	14 (70%)*	13 (65%)	7 (35%)
Partially passable	7 (35%)	4 (20%)	4 (20%)	3 (15%)	4 (20%)	6 (30%)	7 (35%)
Obstructed	9 (45%)	3 (15%)	2 (10%)	0 (0%)	2 (10%)	1 (5%)	6 (30%)

* – significant difference between the groups ($p < 0,05$)

Table 3. Reproductive function of women in 2-24 months after the EP episode

	Group I n=20	Group II n=40		Group III n=80			
		II A n=20	II B n=20	III A n=20	III B n=20	III C n=20	III D n=20
Maternal pregnancy	2 (10%)	12 (60%)	6 (30%)	15 (75%)	14 (70%)	5 (25%)	1 (5%)
Reproductive technologies	3 (15%)	2 (10%)	3 (15%)	1 (5%)	2 (10%)	2 (10%)	3 (15%)

laparotomy tubectomy (Group I), which was the highest indicator in comparison with the whole population. In the group III C(laparotomy with tubectomy after methotrexate therapy), tubal peritoneal infertility was observed in 6 (30%) of women, which is 15% lower than in the group I ($p < 0,05$). In groups II B and III B there was a decrease in the number of tubal obstruction down to 10% and 5% compared to group IIIC, respectively.

Analysis ofthe data presented in Table 3 revealed that spontaneous pregnancy occurred in 2 women (10%) with laparotomy tubectomy (Group I) and in 1 (5%) woman who was performed laparotomy tubectomy and previously used methotrexate (Group III C), however there was no significant difference between these groups.

Pregnancies occurred in 6 (30%) women of group IIB and in 5 (25%) women of group IIIB, which was 20% more than in the case of laparatomy surgery (group I). If the uterine tube is maintained, the percentage of pregnancy in the natural way was almost 2 times higher. The pregnancy occurred in 12 (60%) women in the group IIA, which was 2 times higher than in the group II B and 6 times higher than in the group I. Patients of group III A showed the best result: 15 women (75%) became pregnant independently, which is 15% higher than in the group IIA. In the group III B, when using methotrexate and laparoscopic removal of a fertilized egg with preservation of the fallopian tube, this figure was 70% (14 women), or 5% lower than when using only methotrexate (group III A), but – 10% higher than after conservative removal of a fertilized egg by a laparoscopic method without the use of methotrexate (group II A).16 (11.4%) of women out of total 140 were pregnant with the help of auxiliary reproductive technologies, but differences in groups were not statistically significant, indicating the independence of this factor from the type of treatment of ectopic pregnancy in the history (Table 3).

A new ectopic pregnancy occurred in one woman of group IIA (after the conservative removal of the fetal egg) after 1.5 years. This pregnancy was localized in the opposite tube from the operated one. Two women (10%) in the group I were readmitted with an ectopic pregnancy in a single uterine tube.

An increase in the proportion of ectopic pregnancy in the gynecological hospital was establishedon based on the conducted clinical and statistical analyses of the structure of gynecological diseases from years 2005 to 2015.

The risk factors for developing ectopic pregnancy as follows: early sexual life (OR = 4.36; 95% CI: (1.50-12.69); $p < 0.05$), use of intrauterine helix as a contraception (OR = 6.9, 95% CI: (2.07-22.00), $p < 0.05$), abortions and inflammatory diseases of the uterus and appendages in the history (OR = 2.13; 95% CI: (0.63 -7.16); ($p < 0.05$).

The markers of EP diagnosis and the effectiveness of the drug treatment of this pathology are the dynamic determination of serum β-HCG levels, ultrasound examination of ectopic pregnancy with a transvaginal probe.

It has been established that significant changes have taken place in the approaches to the management and surgical treatment of ectopic pregnancy in the last decade. Thus, in 2005, the cases of laparotomy operations was 86.88%, and medical treatment was only 13.16%. In 2015, the number of patients treated with methotrexate was already more than a half (51.35%) the percentage of laparotomy operations decreased to 18.92%, and the number of laparoscopic interventions with the preservation of the fallopian tube almost doubled.It was also found that restoration of menstrual function in patients receiving methotrexate was later compared to mechanically isolated ectopically placed fatal eggs.

The use of methotrexate before surgery in patients with progressive ectopic pregnancy with high levels of β-HCG (> 1500 IU / L) and a fetal egg of more than 3.5 cm, followed by the conservative removal of a fertilized egg by laparoscopic access, helps to maintain the patency of fallopian tubes in 70% of cases, due to, in our opinion, medication apoptosis of trophoblast cells, separation of trophoblast from the walls of the uterine tube and thrombosis of the attachment site, that may limitmassive coagulation during surgery.

When comparing the results of the patency of the uterine tubes after the use of laparotomic access with laparoscopic access, there was a decrease in the number of tubal obstruction to 10% and 5% respectively. The effectiveness of medical treatment of ectopic pregnancy by methotrexate, which was manifested in the survival of fallopian tubes compared with surgical intervention, was established. If the uterine tube is maintained, the percentage of pregnancy in the natural way was shown to be almost twotimes higher. The best long-term outcome was shown inpatients treated with methotrexate.

CONCLUSIONS

1. Women diagnosed with progressive ectopic pregnancy should be offered conservative medical treatment that mayhelp preserve their reproductive function in the future.
2. Treatment of women with progressive ectopic pregnancy, with an uterine tube size even more than 3.5 cm and unful filled reproductive plans, should include the introduction of methotrexate for 6-24 hours prior to surgery, followed by the conservative removal of the fertilized egg via a laparoscopic access. This approach was shown to maintain the patency of fallopian tubes in 70% of cases.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

HEALTH-SAVING COMPETENCIES IN PHYSICAL EDUCATION OF STUDENTS

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ABSTRACT

The aim: The article examines the health-saving competencies of fitness technologies usage during physical education classes.

Materials and methods: Experimental work was carried out in three stages, each of which was characterized by certain goals and objectives, corresponding forms and methods of research organization. To determine the formation of each of the studied components, we used complex of valid diagnostic techniques.

Results: Implementation of the methodological system significantly influenced on general level of motivational, cognitive, activity and reflexive component formation, which significantly improved, compared with the students of control groups who studied under the traditional system. Evaluation of students' competence in applying fitness technologies showed a high efficiency of the introduced methodological system.

Conclusions: The introduced methodical system of fitness technologies application contributed to increase of the level of students' preparedness by criteria of organizational, communicative, perceptual, speech abilities to fitness technologies, general cultural level, social activity and their involvement in healthy lifestyle. At the same time, the formation of competences in fitness technology usage contributed to the creation of new content of the main components of the methodological system.

KEY WORDS: health-saving competences, criteria, levels, students, physical education, fitness technologies

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INTRODUCTION

The problem of forming, preserving and strengthening the health of student youth is relevant in the contemporary dimension of being. The current society is characterized by a decline in the health of Ukrainians, which needs to be restored.The indicator of the state of human health, which is determined by the length of its life, educational level and absence of physical defects and diseases, has also noticeably decreased. Evidence of this is that in Ukraine during the period of 2010-2018, the quality of life index has decreased from 45 to 75 place and the life expectancy of average Ukrainian by 2018 is 65.1 years, whereas in 2005 it was 69.2 years, which is 10 years less than in the USA and Western Europe, 4 years less than in Poland, 2 years less than in Hungary. Since humanity is concerned about the state of its physical health, there is a need for health-saving and health-improving technologies usage not only in institutions of higher education, creating a health-saving educational environment, but also in everyday life. The use of fitness technology contributes to the formation, preservation and strengthening of students' health, teaches them a healthy lifestyle. Often fitness even provides restoration of lost functionality of the human body. Fitness and health are interconnected by cause-and-effect relationships, and health is the greatest value of a person. The refore, for a healthy and happy life, students need appropriate conditions, a special place among which belongs to the lifestyle. It is important that the latter is properly filled with motor activity, which fitness can provide. The analysis of scientific research and publications shows that the issue of fitness technologies usageduring physical education classes of students has great scientific and practical importance. Scientists emphasize that fitness technology is the most important means of improving the state of health, increasing bodyfunctional capabilities, as harmonious basis ofstudents' physical development. The search for new effective forms and methods of physical education of students is necessary [1; 2; 3; 4; 5; 6].

THE AIM

The purpose of the article is to investigate the health-saving competence of fitness technologies usageduring physical education classes for students.Objectives of the article: 1) to determine the criteria for students' readiness to acquire healthcare-saving competencies; 2) to determine the level of healthcare-saving competencies formation during physical education classes of students.

MATERIALS AND METHODS

Experimental work was carried out in three stages, each of which was characterized by certain goals and objectives, corresponding forms and methods of research organi-

zation. At the initial stage of the research (2015-2016) – search-theoretical – a theoretical analysis of valeological, psychological-pedagogical and methodological literature on the problem of fitness technologies usageduring physical education classes of students was carried out, the general theoretical and methodological foundations of the research were determined; we put forward the working hypothesis and described the general ideas of the organization of research-experimental work. At the second stage (2017-2018) – experimental – a methodical system of fitness technologies application was developed and theoretically grounded duringphysical education classes of students, a comprehensive selection of methods for conducting the research was carried out, experimental work was carried out on approbation of the developed methodical system. The third stage (2018-2019) – generalization – the analysis of research-experimental data was carried out, the general conclusions of the study were formulated; methodical recommendations for the implementation of the developed methodical system were developed. The participants of experiment were divided into two groups (experimental and control) for further comparison of the results from introduction of the developed methodological system. To determine the formation of each of the studied components, a complex of valid diagnostic techniques was used.

RESULTS AND DISCUSSION

The whole complex of psychological and pedagogical measures related to conducting scientific researches was taken into accountduring evaluation of the effectiveness of fitness technologies methodical application during physical education classes.The identification of the effectiveness of fitness technologies methodical application during physical education classes was determined by such criteria as evidences, that show the qualitative and quantitative effectiveness of educational work and, in particular, the assessment of applied fitness technologies in the process of training student youth. In general, the effectiveness of the methodical system was evaluated according to specific indicators, which were intended to improve the quality of physical educationprocess, the level of health, physical development, physical preparedness and readiness of students to use fitness technology in their further life.

Under the efficiency of the functioning of physical education methodical system G.P. Griban, besides mastering the general tasks of physical education, considers the appearance of physical culture and health competencies as some internal, potential, hidden psychological neoplasms: knowledge, motives, preferences, interests, abilities, skills, programs of actions, systems of values, attitudes to physical culture and health activities that are manifested in the methodological competence of introduction and transfer to other members of society, in particular in the process of professional activity [7, p. 358].

It should be noted that the important condition for students' competenceformation in fitness technology usage is to ensure continuous self-education. The focus of the students on self-education and self-development in the pedagogical, general cultural and personal plan is an important indicator of teachers from physical education departments ability to focus on improving the quality of the educational process of physical education [8; 9]. Formation of students' competencies regarding fitness technologies usage took place, while taking into account the practical conditionality and significance that is why they are necessary for the student in his further life. Under competencies it is considered a dynamic combination of knowledge, skills and practical abilities, ways of thinking, professional, ideological and civic qualities, moral and ethical values, which determine the ability of a person to successfully carry out professional and educational activities and is the result of training at a certain level of higher education [10, p. 28-29].

Health-saving competencies are the readiness of a graduate from educational institution to the tests that we face in public life, natural conditions; the ability to independently solve problems regarding the preservation of their own health, as well as increasement of healthy life duration through the system of modeling and predicting ways of using both physical culture means and possible changes in their own health. Physical education can be interpreted as a form of manifestation and the formation of competencies [11; 12; 2; 13; 14; 15; 16; 17 etc.]. The level of health-saving competencies of students in physical education is determined by readiness to use fitness technologies, which serves as an indicator of students' education in matters of maintaining their own health. The ability of students to maintain, improve and maintain their own health is a superior expression of their competence. Competencies can be considered as indicators that determine the readiness of a future specialist for further life and personal self-improvement in society and in the context of future professional activities [7; 10; 6; 18].

The main indicator of the effectiveness of fitness technologies methodical application implementation during physical education classes is the presence of physical culture and health competencies in students. Criteria of students' readiness to master competencies during fitness technologies usage were defined motivational, cognitive, activity and reflexive components that were evaluated for high, sufficient, medium and low levels of manifestation.

Implementation of the methodological system significantly influenced the general level of formation of the motivational component, which significantly improved compared with the students of control groups (CG) who studied under the traditional system. Thus, the high level of competence formation in experimental groups (EG) was: in men – 25,53%, in women – 21,13%; sufficient level of competence in men – 34.04%, women – 32.39%; the average was 31.92% and 36.62%respectively, low level in male group was in 8.51%, in female was 9.86% (Fig. 1).

In the control groups that were engaged in the traditional system of physical education, the high level of competence development of application of fitness technologies is observed only in 2.44% of males and 2.74% of females, sufficient 17.07% of males and 12,33% of females respec-

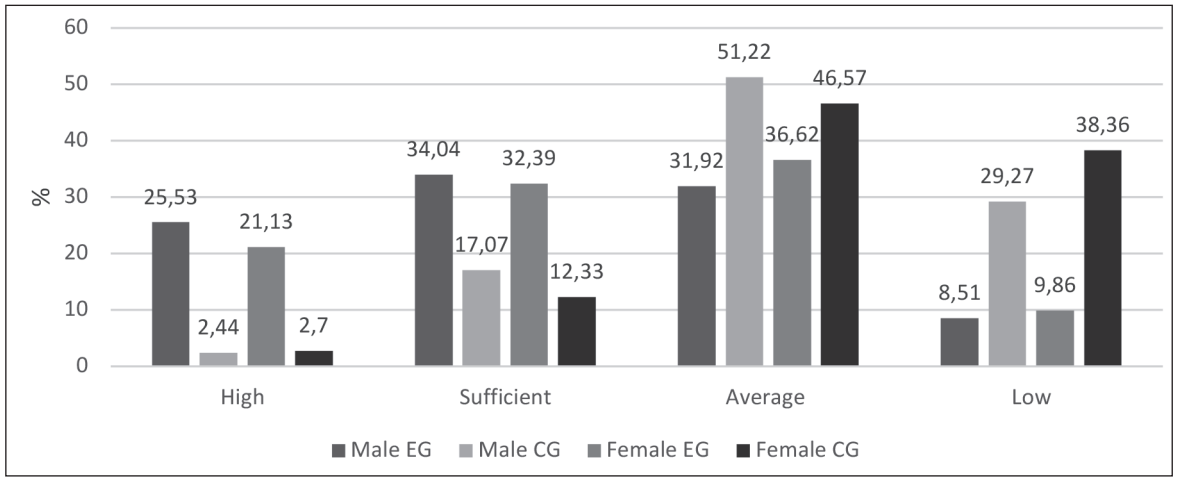

Fig. 1. Levels of training of students from experimental groups on the fitness technologies application competence based on motivational component

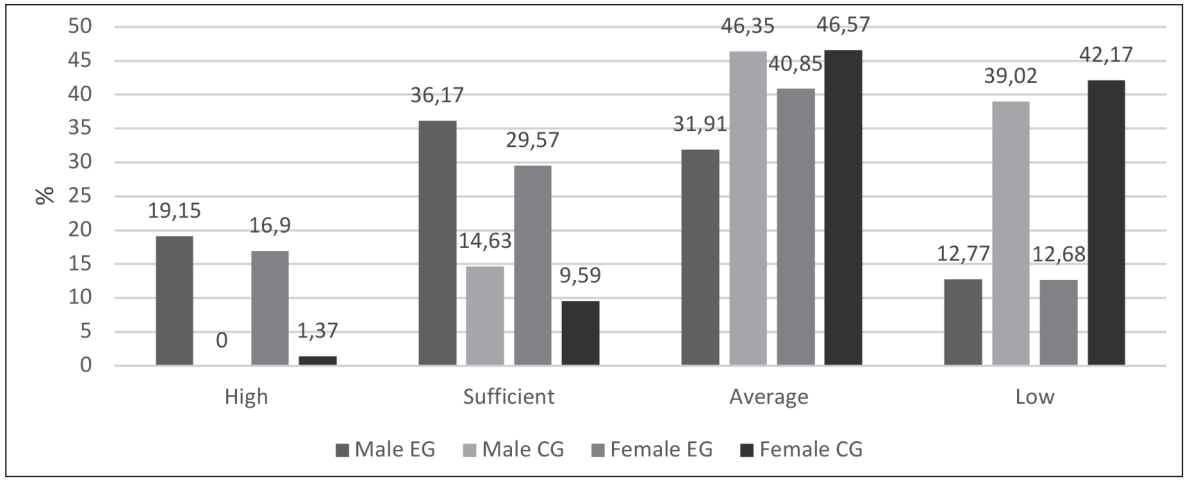

Fig. 2. Levels of training of students from experimental groups on the fitness technologies application competence based on cognitive component

tively, average in 51.22% and 46.57%, low in 29.27% and 38.36%.The obtained data shows a significant difference in competence formation of students from control and experimental groups, which confirms the effectiveness of the implementation of the methodological system during physical educationclasses.

Assessment of acquired competencies by students with the help of cognitive component during studying byfitness technologies methodical system application and traditional system of physical education revealed a positive dynamics regarding acquired knowledge and skills necessary for the organization and implementation of physical education and health measures for the introduction of fitness technology only in experimental groups. The formation of competencies in the cognitive component was evaluated by the presence knowledge and skills, the availability of methodical arsenal and methods, organizational skills, the ability to conduct physical culture and recreational activities, etc.

We found out that a high level of competence in the cognitive component was in 19.15% of men and 16.90% of women in experimental groups. In control groups in men, no student with a high level of competence was found, and in women only 1 student, which is 1.37%.A similar tendency persists during the analysis of the competence of students with a sufficient level, in experimental groups – 36.17% of men had this level and 29.57% women; in control groups 14.63% and 9.59%respectively, which significantly indicates the different levels of preparedness of students from experimental and control groups.The number of studentswith the average level of competence grows at the expense of their release from a low levelin experimental groups, while in control groups, on the contrary, their number increases due to the high and sufficient levels (Fig. 2). Significant difference is observed in low-level competence group, where significant differences between experimental groups were found: men – 12.77%, women – 12.68%, and control groups – 39.02% and 42.47% respectively.

The general level of competence development by cognitive component of fitness technologies application of students from experimental groups has positive dynamic after introduction of the methodical system during physical education classes. At the same time, such a form of conducting the discipline "Physical education" in general positively affects the formation of world outlook and the general cultural level of student youth.

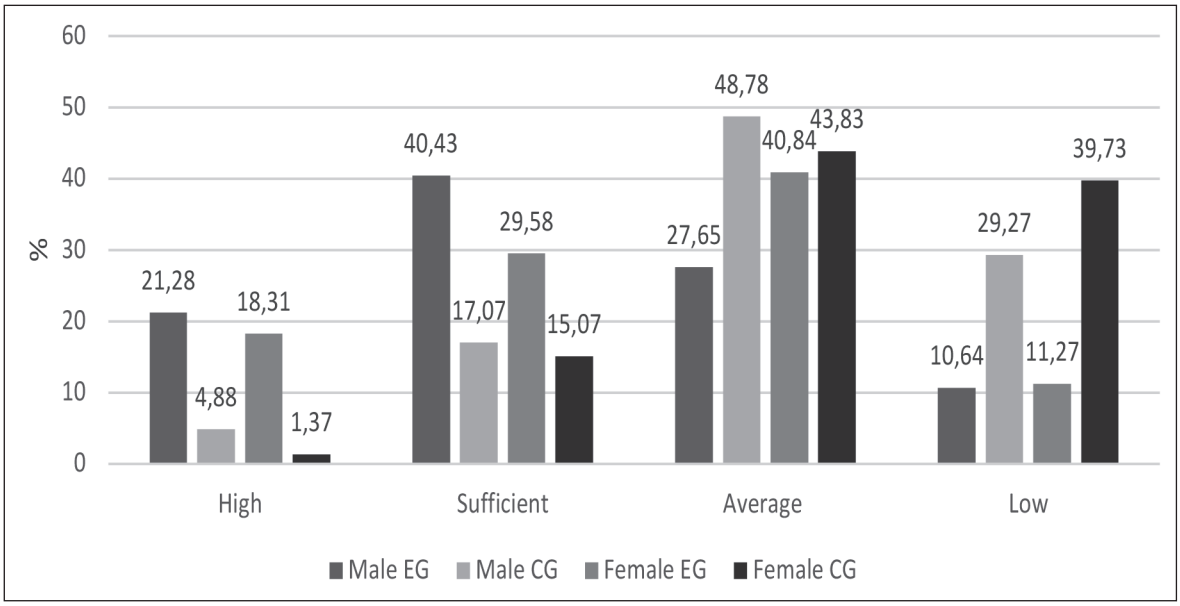

Fig. 3. Levels of training of students from experimental groups on the fitness technologies application competence based on activity component

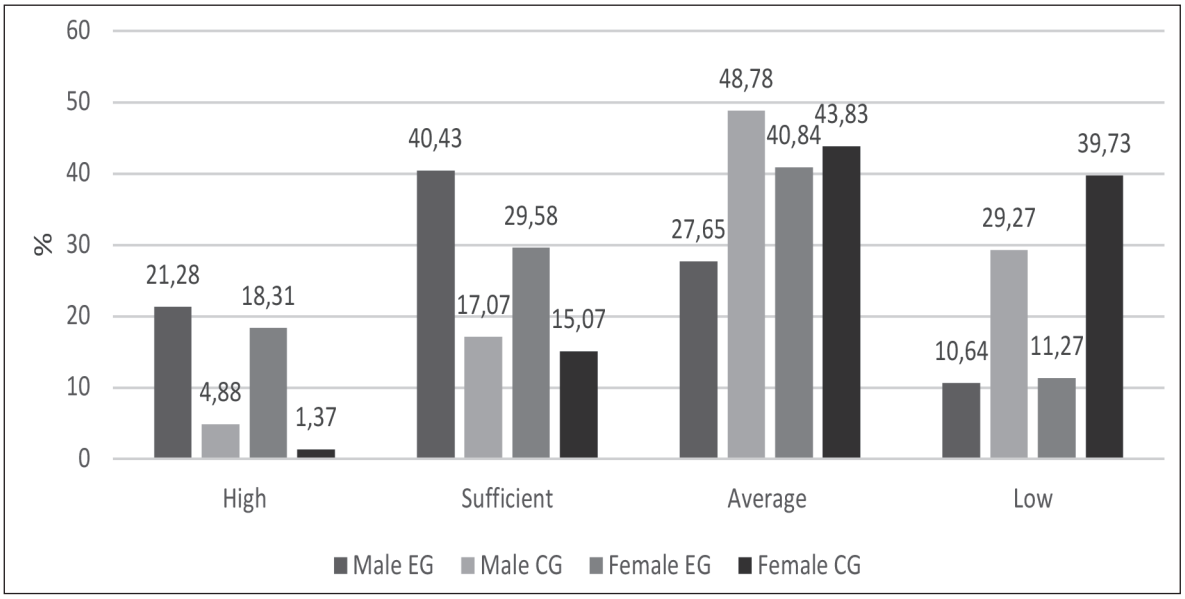

Fig. 4. Levels of training of students from experimental groups on the fitness technologies application competence based on reflexive component

Conducted control assessment after implementation of the methodological system by the activity component, confirms the improvement of the results of experimental groups with a high level of competence: EG (male) – 21.28%, EG (female) – 18.31%; with sufficient level: EG (male) – 40,43%, EG (female) – 29,58%; with an average: men -27.65%, women-40.84%; and low – 10.64% and 11.27% respectively (Fig. 3). In control groups where the methodical system of application of fitness technologies was not implemented, the indicators are much lower. With a high level of competence: CG (male)– 4.88%, CG (female)– 1.37%; with sufficient level: CG (male) – 17.07%, CG (female)– 15.07 %; with an average: men – 48.78%, women-43.83 %; and low – 29.27 % and 39,73 % respectively.

Evaluation of students' competence in applying fitness technologies by reflexive component showed a high efficiency of the introduced methodological system. Thus, with a high level of competence in experimental groups were 17.02% of men and 12.68% of women and,at the same time, among students from control groups, who were engaged in the traditional system of physical education, no student was found with high level of competency. Significant discrepancies are observed when comparing students from experimental and control groups with a sufficient levelof competency, namely: among men we detected 40.42%, among women – 32.39%, in control groups thereweresignificantly lower number of such students – 19.51% and 16.43% (Fig. 4).Male students with an average level of competence in experimental groups are lowerbecause increase in higher levels (sufficient and high level of preparedness), in female groups, the difference is not significant. At the same time, both male and female control groups have a fairly large difference in

number of students with a low level of competence compared with experimental groups.If in experimental groups of men, only 12.77% of students have a low level of competence, then in similar control groups such students were 29.27%, women showed – 12.68% (EG) and 39.73% (CG).

Students from experimental groups have significantly better indicatorsby the level of assimilation of special knowledge, skills and abilities to exercise control over physical fitness, state of health, ability to apply in practice new fitness technologies, means and methods of development of physical qualities. The introduced methodical system of fitness technologies application contributed to increase of preparedness level of students from experimental groups according to criteria of organizational, communicative, perceptual, speech abilities to fitness technologies, general cultural level, social activity and their attraction to a healthy lifestyle. Competenciesformation in fitness technology usage contributed to the creation of new content in main components of the methodological system.

CONCLUSIONS

Thus, timely and qualitative diagnostics of the current physical state of students, the level of their physical and functional preparedness is important for fitness technologyusage in further life. Meanwhile, existing methods of estimation of these parameters today, connected mainly with conducting special motor tests, are no longer able to provide the teacher with clear and accurate information and require qualitative research, which makes it difficult to obtain operational information during the course. Another important criterion for evaluating the competencies of personal self-improvement is the ability to assess self-development, self-improvement, learn emotional self-regulation, ability to choose effective ways, means, methods, techniques and forms of motor activity in personal interests and opportunities, which should promote continuous physical perfection, adherence to a healthy way life, self-knowledge, development of physical qualities, formation of special skills and abilities. Prospects for further research are aimed at the study of theoretical and methodological principles of vocational training of physical education department teachers for physical education and health activities of students.

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ORIGINAL ARTICLE
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SPECIALTIES OF HYSTOMORPHOMETRICAL CHANGES IN PLACENTA OF WOMEN WITH EARLY AND LATE PREECLAMPSIA

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ABSTRACT

The aim: To find out typical pathomorphological differences in placenta of women with early and late preeclampsia.

Materials and methods: Investigation includes 40 placentas from deliveries in women with preeclampsia (main group) and 40 placentas from physiological delivery in somatically healthy women, who had no complications during pregnancy (control group). Placentas in the main group were devided into two sub-groups (20 in each) – with early and late preeclampsia. Specialties of the blood vessels in normal pregnancy were investigated, and their structural transformation with the developement of preeclampsia, according to the appearence of perinatal pathology. Morphometrical data of the blood stream was investigated with the help of eyepiece and program Image Tools 3,6.

Results: Significant decrease of weight ($p<0,05$), square and volume of placenta was common to early preeclampsia, comparing to the same characteristics in late Preeclampsia (PE). Specific gravity of villi without vessels, hardened blood vessels, hardened villi and fibrinoid altered vessels was increased statistically significantly ($p<0,05$) in placenta of women with early PE, comparing to women with late PE. The number of effective blood vessels crossings was determined mostly in late PE, comparing to the early form ($p<0,05$). Found out significant defferences ($p<0,05$) in changes of hystovasoarchitecture of placenta in early preeclampsia, according to the number of immature villi and villi with no signs of compensatory angiomatosis.

Conclusions: Increased number of hypoplasia of placenta, breach of effective placental blood stream and significant decrease of compensatory and adaptive changes in placenta are more common to early PE, comparing to late PE.

KEY WORDS: late preeclampsia, placenta, gestational endotheliosis

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INTODUCTION

Preeclampsia (PE) became the complication of 2-8% pregnancies in the world, PE remains one of leading reasons of maternal (50 000 cases annually) and perinatal morbidity and mortality. Perinatal morbidity for this pathology hesitates in limits from 10 to 30 ‰, perinatal morbidity – from 463 to 780 ‰[1,2]. PE is characterized by increase of systolic blood pressure ≥ 140 mmHg and diastolic BP ≥ 90 mmHg, measuring conducted twice with an interval at least 4 hours. Without regard to the appearence of pathophysiological changes (violation of process of placentation) at the beginning of pregnancy, hypertension and proteinuria, as a rule, show up only in the second half of pregnancy and present in 2-12% of all cases of pregnancy. The symptoms of PE can embrace the general symptoms of hypertension and / or proteinuria, such as headache, delay of intrauterine growth of the fetus, nausea, vomiting, visual inpairment and edema [3]. In the second and at the beginning of third trimester of pregnancy the treatment of PE is mostly symptomatic, with the aim of normalization of blood pressure and warning of neurological symptoms. In groups with high risk, small decline of risk of development of PE is achived by taking 100 mg of acetylsalicylic acid from 12 weeks of gestation and calcium (1g of elementary calcium in twenty-four hours, from 16 weeks of pregnancy). However, a primary prophylaxis of PE is the a reason of many disscusions and active scientific researches within the framework of that the special attention is payed to the assignment of antiplatelet drugs and vitamin D. Well-proven clinical unefficiency of vitamins-antioxidants C and E, precursors and donators of oxide of nitrogen, polyunsatured fatty acids for warning the development of hypertension during pregnancy [3,4]. The only undoubtedly effective method of treatment of PE is termination of pregnancy or delivery, although for some women with preeclampsia there can be temporal worsening of condition in postpartum. Grant of help to the women with PE has for an object minimization of the further complications related to pregnancy, prevention of preterm delivery and increase of number of favourable results for mothers and newborn.

In physiological pregnancy the tissue of placenta gets to the uterine arteries of mother that results in the decrease of elasticity of arteries and increases uteroplacental blood flow. In early preeclampsia (<34 weeks) this invasion is insufficient, in the result ischemia of placenta develops and nonoptimal haemodynamic providing of pregnancy. Late PE (≥ 34 weeks) is characterized by greater degree of metabolic violations in the organism of mother, than by an inadequate placental invasion. In both cases as a result development of gestational endotheliopathy, inflammatory maternal immune answer, hypertension and damage of

vessels in kidneys, liver and brain [5,6,7]. The increase of placental invasion and intimacy of contact between mothers and fetus are related with the increase of rates of perinatal growth (but not absolute size) of cerebrum and body of the fetus [7]. Early development of PE has the most unfavorable consequences, as for a mother so for the fetus. Many pathomorphological changes appear in early terms of pregnancy, before clinical symptoms.

THE AIM

To find out typical pathomorphological and hystomorphometrical differences in placenta of women with early and late preeclampsia.

MATERIALS AND METHODS

On histological research 40 placentas were taken from physiologycal deliveries from somatically-healthy women , with pregnancy without complications (I group – is a control group) and 40 placentas from deliveries from women with PE (II –main group that was divided into two sub-groups (20 placentas in each sub-group) – with early PE, that developed before 34th week of pregnancy and late PE, that developed after 34 weeks of pregnancy), analysed features of vascular system in norm and features of its structural changes in connection with the development of preeclampsia in different terms.

Pathomorphological research was conducted in extremely early terms after delivery according to the generally accepted methodology. The pieces of placentas were cut out from central, paracentral and regional parts, through all layers of placental tissue, in an amount 8, by sizes 1,0x0,5x0,5 cm, from an umbilical cord 2 pieces were cut out – in the distance of 2 cm from the place of attachment of umbilical cord to the placenta and on an opposite side.

Taken material was fixed by 10% – water solution of neutral formalin not less than 48 hours, it was washed then, dehydrated in the system of polyhydric alcohols, poured in paraffin blocks according to a standard scheme. Prepared semithin cuts, thickness 5-7 mcm, were dyed with hematoxylin and eosin and conducted painting after van Gieson, after that conducted the microscopy of the prepared histological preparations by means of light microscope OLIMPUS BX41 increases in 100, 200 and 400 times. Determinations of morphometric parameters of the bloodstream carried out by means of eyepiece, that contains 49 squares, the area of each square is $3{,}45x10^{-4}$ мм^2 at an increase 269,2 and program Image Tools 3,6. The estimation of statistical authenticity of difference of quantitative indexes was executed by means of the program Microsoft Excel. For the further processing of data such statistical methods were used, as getting of mean values and RMS errors, student t-test number.

The study was approved by the Ethics Committee of the Vinnytsya Pirogov Memorial National Medical University.

RESULTS

The middle term of delivery in the control group was 35,1±2,6 weeks (women had 32 – 40 weeks of pregnancy). All organometrical parameters – linear sizes, weight and area of maternal surface, were according to the term of gestation. At overview histological research in the cases of normal pregnancy in the general mainly took place involutive and degenerative changes in placental tissues, as moderate fibrosis of stroma and vascular walls of stem and intermediate villi, laying of particles of fibrinoid in a moderate amount at intervillous space and stroma of part of stem villi (signs of physiological maturity of placenta), in some placentas compensatory and adaptive mechanisms took place, such as local compansatory angiomatosis of intermediate and terminal villi (38,2 %), their budding(34,3%). In the cases of preterm delivery, the signs of immaturity of placental tissue and compensatory and adaptive mechanisms took place as local compansatory angiomatosis of intermediate and terminal villi(42,3 %), their budding (54,8 %).

All organometric parameters – linear sizes, weight and area of maternal surface in part of cases were according to the term of gestation, sometimes placentas had less morphometric values then it should have been, hypoplasia of placentas took place. During overview histological research in the cases of the full-term pregnancy next to general involutory-degenerative and compensatory-adptive changes in placental tissue, took place mildly expressed pathological changes. The signs of immaturity of placental tissue took place in the cases of preterm deliveries, low compensatory-adptive mechanisms, and pathological changes were considerably expressed.

It was set from data of morphological research, that placentas, in II (main) group – I sub-group (early PE)had round and oval forms, however there were placentas of extended irregular shape, three-cornered form, placentas with additional parts and double-particle placentas. Middle weight of placentas in this group was 312,4 ± 23,2 g, area of maternal surface 172,3 ± 13,2 cm^2, volume 289,8 ± 8,4 cm^3, placenta-fetus coefficient (PFC) was 0,1±0,02 (table. 1).

The analysis of organometric parameters of placentas in this group showed that in 25% of cases (5 placentas) was found hypoplasia of placenta. Considerable pathological changes were determined on fetal surface. On some placentas the roller of different width took place on the edge, part of placentas had a rim, focal compressions located on the edge, close to the central or in central part of fetal surface. Also the haematomas of different sizes and term, phlebeurysm, took place in some placenta. Unlike in control group, attachment of umbilical cord was mainly regional and thecal with the intermediate and loose type of branching of vessels. Almost in the half of cases hypersinuosity of umbilical cord took place with varicose expansion of veins (43,2 %), the hypoplasia of Whartons jelly took place on occasion, vessels were located on the edges of umbilical cord, easily collapsed. The transversal crossing of some cords had not round, but extended form, here vessels were located not in triangle form, but in one row and had not round, but extended form.

At the review of maternal surface it was whole, or had defects, with badly expressed cotyledonies, widespread with blood cloats of different term (signs of the partial preterm

Table 1. Macromorphometric parameters of placentas of women in main and control groups

Parameters	Main group, I subgroup, n=20	Main group, II subgroup, n=20	Control group, n=40
Weight of placenta, g	312,35 C 23,14*	405,48 ± 22,34	472,45 ± 34,18
Area, cm2	172,26 ±13,18**	194,12 ± 14,05	268,72 ±16,24
Volume, cm3	289,78 ± 28,4**	332,68 ± 29,2	433,38 ± 31,3
PFC	0,1 ± 0,02	0,12 ±0,02	0,18 ± 0,04

Notes: * - differences are statistically reliable (p <0,05) in relation to analogical indexes of control group;
** - differences are statistically reliable (p <0,05) in relation to indexes for women with late preeclampsia.

Table 2. Micromorphometric parameters of placentas of women in main and control groups

Parameters	Main group, I subgroup, n=20	Main group, II subgroup, n=20	Control group, n=40
Number of the effective crossings of vessels	2984516,26 ± 5512,32**	3534663,34 ± 6732,14*	5692318,74 ± 7886,28
Specific gravity of avascular villi, %	14.85 ± 0,78**	10,45 ± 0,66*	4,52 ± 0,22
Specific gravity of sanguineous villi,%	6,54 ± 0,34**	16,12 ± 0,48*	27,36 ± 1,02
Specific gravity of sclerotic vessels, %	36,47 ± 3,4 **	21,32 ± 2,4*	11,05 ± 1,3
Specific gravity of sclerotic villi, %	38,14 ± 3,3**	28,32 ± 3,1*	13,56 ± 2,2
Specific gravity of fibrinoid-changed villi,%	18,64 ± 1,8**	9,48 ± 1,1*	4,28 ± 0,6

Notes: * - differences are statistically reliable (p <0,05) in relation to analogical indexes of control group;
** - differences are statistically reliable (p <0,05) in relation to indexes for women with late preeclampsia.

placental abruption), hemorragic and ischemic strokes of different localization took place and in different amount, numeral calcinates in decidual plate and in placental tissue. Also the cavities filled with blood, were determined in the layer of placenta tissue.

From data of morphological research of placentas in II sub-group(late PE) of the main group, they had round and oval forms, among that there were placentas of extended irregular-shape, three-cornered form, placentas with additional parts and double-particle placentas. Middle weight of placenta in this group was 405,5± 22,4 g, area of maternal surface – 194,1± 14,1 cm^2, volume of 332,7 ± 9,2 cm^3, PFC was 0,12±0,02 (table. 1) on the average. The analysis of organometric parameters of placenta in this group showed that 10% cases (2 placentas) had hypoplasia of placenta. In some placentas there was a roller of different width on the edge, part of placentas had a rim, focal compressions located on the edge, close to the central or in central part of fetal surface. Also the haematomas of different sizes and term, varicose expansion of vessels, took place in placentas. Attachment of umbilical cord was mainly central or eccentric, in some placentas regional and thecal, with the intermediate and loose type of branching of vessels. Only in some cases hypersinuosity of umbilical cord took place, with varicose expansion of veins (in 3 placentas – 15%), the hypoplasia of Whartons jelly took place in some cases, vessels were located on the edge of umbilical cord, that easily collapsed. The transversal crossing of some cords had not round, but extended form, here vessels were located not in form of triangle, but in one row and had not round, but extended form.

At the review of maternal surface it was whole, or had defects, however less than it was expressed, comparing to changes in placentas from women with early preeclampsia.

At histological research of placentas of women with preeclampsia of different degree and forms almost in all cases (89,3%) violation of maternal and fetal bloodstream was diagnosed, as acute and chronic hemorragic (26,2%), and also ischemic (38,2%) strokes, widespread hemorrhages at intervillous space (19,1%). The presence of small "old" haematomas on the edge of placentas was testified to their partial regional preterm abraption (11,3%). In some cases (7,3%) the thrombosis of intervillous space was determined, mainly in the central zones of placenta. Also there was considerable laying of particles of fibrinoid and fibrin in intervillous space and basale plate, in the places of necrosis of epithelium of villi (42,1%), with formation of widespread zones of fibrinoid necrosis. Because of expressed degenerative changes, as a result of considerable disorders of utero-placental bloodstream in placentas, compensatory-adaptive mechanisms developed as focal hyperplasia of terminal villi with their budding(14,4%), with focal compensatory angiomatosis (hyperplasia of capillaries) of terminal villi(11,3%), but comparing to placentas of control group the processes of compensation and adaptation were extremely low.

Also at histological research of placentas such microscopic parameters of microvasculare system, as a number of the

Table 3. Micromorphometric parameters of placentas of women of main group at early and late preeclampsia

Pathomorphological changes	Early preeclampsia, n=20, n (%)	Late preeclampsia, n=20, n(%)	p	RR, 95% CI
Laiyng of fibrinoid at intervillous space	12 (60,0)	8 (40,0)	0,21	1,5 (0,79-2,86)
Trombosis of vessels of villi tree	7 (35,0)	4 (20,0)	0,30	1,75 (0,61-5,05)
Trombosis of intervillous space	4 (20,0)	1 (5,0)	0,19	4,0 (0,49-32,73)
Immature villi	18 (90,0)	5 (25,0)	0,001	3,6 (1,66-7,80)
Villi with sclerotic, fibrinoid-changed stroma	7 (35,0)	2 (10,0)	0,09	3,5 (0,83-14,83)
Fresh Hemorrhagic strokes	8 (40,0)	2 (10,0)	0,06	4,0 (0,97-16,55)
Old Ischemic strokes	5 (25,0)	1 (5,0)	0,13	5,0 (0,64-39,06)
Laiyngs of big calcinates	4 (20,0)	2 (10,0)	0,39	2,0 (0,41-9,71)
Villi with signs of compensatory angiomatosis	4 (20,0)	11 (55,0)	0,04	0,36 (0,14-0,95)
Ammount of terminal and intermediate villi with features of budding	3 (15,0)	9 (45,0)	0,06	0,33(0,105-1,05)
Trombosis of umbilical cord vessels	6 (30,0)	1 (5,0)	0,08	6,0 (0,79-45,42)

effective crossings of vessels, specific gravity of avascular villi, specific gravity of sanguineous villi, specific gravity of sclerotic vessels, specific gravity of sclerotic villi and specific gravity of fibrinoid-changed villi, were determined.

From results given by us in a table it's evident, that at early PE there were expressed changes in the vessels of microvasculare system, namely, an amount of the effective crossings of vessels at early PE was almost in 1,5 times less than, at late PE and 2 times less than, in the placentas of women who had physiologycal pregnancy. More than three times, specific gravity of avascular villi grew in the cases of early PE comparing to normal deliveries and 1,5 times more as compared to late preeclampsia. Specific gravity of sanguineous villi in a control group 4 times higher comparing to a main group, that can testify to the considerably higher index of bloodstream in normal placentas. In the cases of preeclampsia specific gravity of sclerotic villi and specific gravity of sclerotic vessels in villi was three times higher than the same index at physiological gestation. Also the amount of fibrinoid-changed villi was 4 times higher in the cases of perinatal pathology, with a reliable difference between the sub-groups of pathology ($p < 0,05$).

Thus, from pathological processes in placentas from two comparative groups is diagnosed connection of disorders of utero-placental circulation with general dystrophic changes in villi, as a result of violation of microcirculation. It follows, that in placentas at early PE considerably more often there were disorders of utero-placental circulation, such as hemorragic strokes, focal thrombosis and stasis at intervillous space of subbasale and central zones. Dystrophic changes with the sclerosis of stroma of villi and laying of fibrinoid at villi's stroma, and intervillous space with the development of large focal fibrinoid necrosis, was clearly determined at early preeclampsia in moderate and heavy degrees, as a result of violation of microcirculation in villi of all calibers.

At research of histological preparations, such pathological changes were educed in the structure of placentas, as a fall of fibrinoid at intervillous space. At the early PE a similar finding was more evident (60,0%) comparing to late preeclampsia (40,0%), RR 1,5, CI of 95%, 0,79-2,86, p=0,21 (table. 3). Also we investigated the cases of thrombosis of vessels of villi tree, which one at the early PE had a greater value – 35,0% cases, and at late PE – 20,0%, however the difference between indexes that were investigated, also did not have signs of authenticity, RR 1,75, CI of 95%, 0,61-5,05, p=0,30. The thrombosis of intervillous space took place at early PE in 20,0 %, and at late – 5,0% cases, RR 4,0, CI of 95%, 0,49-32,73, p=0,19. Immature villi were determined more often in placentas from patients with the cases of early PE (90,0%), than in late PE(25,0%), RR 3,6, CI of 95%, 1,66-7,80, p=0,001.

Next to the changes listed above structural violations, there took place structural changes in villi of different caliber, namely it was presence of fibrinoid-changed and sclerotic stroma, that were 3,5 times higher in the cases of early PE, comparing to late PE – 35,0% against 10,0%, RR 3,5, CI of 95%, 0,83-14,83, p=0,09.

We determined fresh hemorrhagic and old ischemic strokes in placentas, their specific gravity was also higher at early preeclampsia – 40,0% and 25,0% against 10,0% and 5,0% at late PE, however marked differences also were not statistically reliable (RR 4,0, CI of 95%, 0,97-16,55, p=0,06 and RR 5,0, CI of 95%, 0,64-39,06, p=0,13).

Severity of compensatory-adaptive mechanisms in the cases of early preeclampsia was statistically less expressed – the amount of villi with the signs of compensatory angiomatosis here was 20,0% against 55,0% at late PE, RR 0,36, CI of 95%, 0,14-0,95, p=0,04. Amount of intermediate and terminal villi with the signs of budding with syncytial knots at early PE was 15,0%, at late PE – 45,0% cases, RR 0,33, CI of 95%, 0,105-1,05, p=0,06. At the same time next to changes in placental tissue pathological changes took place also at the umbilical cord, such as thrombosis of vessels, specific gravity of which at early preeclampsia was 30,0%, at late – 5,0% cases, RR 6,0, CI of 95%, 0,79-45,42, p=0,08.

DISCUSSION

Preeclampsia is a multisystem disease of multivariable genesis including imperfect placentation, oxidizing stress,

autoimmunity, activating of thrombocytes and thrombin, intravascular inflammation, disbalance in angiogenesis, violation of cardiovascular activity of mother and gestational endotheliopathy – as triger of PE. Insufficient invasion of trophoblast can lead to elemination of embryo already in the first weeks of pregnancy, or, in case of progressing of pregnancy, to limitation of growth abilities of placenta, such as her hypoplasia with further development of different obstetric complications [6]. An imperfect placental invasion for certain is related to most cases of early and heavy PE. However, imperfect placentation is less important for development of PE, that shows up later, after 34 weeks. According to literature, comparing to early preeclampsia, at late PE, placenta has considerably low frequency of histological violations, however, maternal factors (for example, metabolic syndrome or chronic hypertension) have a greater meaning. Differences between the early and late stage of PE are also observed in risk factors, maternal vascular reactivity, efficiency of screening and efficiency of prophylaxis.

We found substantial changes of placental histovasoarchitecture, that probably resulted in the decline of perfusion at the early beginning of preeclampsia comparing to physiological pregnancy. Indicated by us corresponds previous ultrasonic research of violations of blood flow in uterus arteries at early preeclampsia [3] and to thistopathological conclusions about the increase of amount of imperfect placentation in women with early PE [7]. In addition, as it shown in previous researches, kids born from mothers with early preeclampsia, had significantly less weight at birth comparing to the babies born from normotensive women in similar gestational age [7], what can indicate on violation of nutritional function of placenta.

The results of our research correspond previous researches, which indicated that late PE is connected with general hypoperfusion [1,5], while early PE is connected with hypoperfusion [5]. Brought up researches also showed the increase of cardiac output in combination with the decrease of peripheral resistance in women who developed late preeclampsia [5]. On the other hand, in women who developed early PE, the decrease of cardiac output and increase of peripheral resistance was diagnosed [1,5].

Thus, conducted histomorphometric researches of preparations confirmed dependence of changes of structures of placenta to the form of preeclampsia. For early preeclampsia lowerness of indexes of compensatory-adaptive mechanisms was common, and extremely heavy pathological changes in tissue of placenta that probably resulted in violation of the haemodynamic providing of pregnancy. As the amount of participants in this research is small, it can be, that the differences found can be conditioned by mixed factors.

CONCLUSIONS

Lower weight ($p < 0,05$), area and volume of placenta was common for early preeclampsia comparing to analogical indexes at late PE.

Violation of effective vascular component at early preeclampsia was met, statistically ($p < 0,05$) more often, than at the late form of PE.

During comparative micromorphometric research there were found reliable differences ($p < 0,05$) in early preeclampsia – amount of immature villi and villi with the signs of compensatory angiomatosis, than at late preeclampsia.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

CYTOKINE AND IMMUNOGLOBULIN SPECTRA OF TISSUE EXTRACTS FROM TONSILS OF CHILDREN WITH HYPERTROPHY AND CHRONIC TONSILLITIS

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ABSTRACT

The aim: To compare the content of α and γ–interferons, interleukins 1β ,4, 10, IgA, IgG, as well as the level of the general forms of immune complexes in tissue extracts from tonsils of children with hypertrophy and chronic tonsillitis.

Materials and methods: We studied tonsils of 25 children aged 5-12 years with hypertrophy of palatine tonsils (HPT) and with chronic tonsillitis (CT). The content of α and γ – interferons, interleukins 1β, 4, 10, IgA, IgG in tonsil extract was determined by immunofluorescence assay. Immune complexes were determined using sedimentary test (3.75% solution of polyethylene glycol).

Results: In tissue extracts from tonsils with CT, there is a predominance of inflammation factors, potential sensitization, and the development of immunopathological reactions. The presence of inflammation is indicated by elevated levels of interleukin-1β, immunoglobulin G. High levels of interleukin-4 may indicate that both HPT and CT have a tendency to increase sensitization to microbial and other antigens.

Conclusion: The results indicate a significant difference in the qualitative and quantitative state of inflammation factors and allergy in case of HPT and CT. In tonsils with CT, there predominate both simple and allergic inflammations, as well as immunopathological reactions.

KEY WORDS: Hypertrophy of palatine tonsils, chronic tonsillitis, inflammation factors, allergy

Wiad Lek. 2020;73(1):156-160

INTRODUCTION

Currently it is clinically proven and experimentally grounded that the structures of Waldeyer's ring have a great impact on the local humoral immunity, both in the nasopharynx and oropharynx, where the primary protection components are secretory immunoglobulin A, interferons and innate immune humoral factors [1, 2, 3, 4]. At the same time, it should be noted that the opinion of various researchers regarding the content of these humoral factors in case of chronic tonsillitis and hypertrophy of palatine tonsils significantly differ. Some authors find with little to no difference in the content of various immunity factors in the tonsillar tissue between hypertrophy and tonsillitis with the exception of tissue morphology [5] and the presence of an allergic background, a significant reactivity change in case of hypertrophy [6]. Other authors believe that hypertrophy of palatine tonsils and tonsillitis significantly differ in quantitative characteristics in the content of factors of nonspecific immunity and inflammation [7, 3]. Therefore, it seems appropriate to further study the humoral protection mechanisms that are in the structures of the pharyngeal lymphoid tissue ring. Thus, the aim of the study is to compare the content of α and γ–interferons, interleukins 1β ,4, 10, IgA, IgG, as well as the level of the general forms of immune complexes in tissue extracts from tonsils of children with hypertrophy and chronic tonsillitis.

THE AIM

The aim of the study is to compare the content of α and γ–interferons, interleukins 1β ,4, 10, IgA, IgG, as well as the level of the general forms of immune complexes in tissue extracts from tonsils of children with hypertrophy and chronic tonsillitis.

MATERIALS AND METHODS

We studied the content of these factors in extracts from tonsils of 2 groups of children aged 5-12 years. Group 1 involved children with hypertrophy of palatine tonsils (HPT) of grade 2-3 (11 persons). Group 2 consisted of children with chronic tonsillitis (CT) (14 persons).

The tissue extracts from tonsils were prepared in a 0.85% NaCl solution at a ratio of 1:5. The technique of preparation of extracts met the recommendations of [8]. Statistical processing was carried out using Mann-Whitney U-test (Wilcoxon) [9]. Immune complexes were determined using sedimentary test with a 3.75% solution of polyethylene glycol according to the recommendations of Nasonov [10]. Determination of interferons and interleukins in extracts was carried out using the Cytokin LLC reagent kits (RF) and the Lab line (Austria) enzyme immunoassay analyzer, and IgA and IgG monomeric

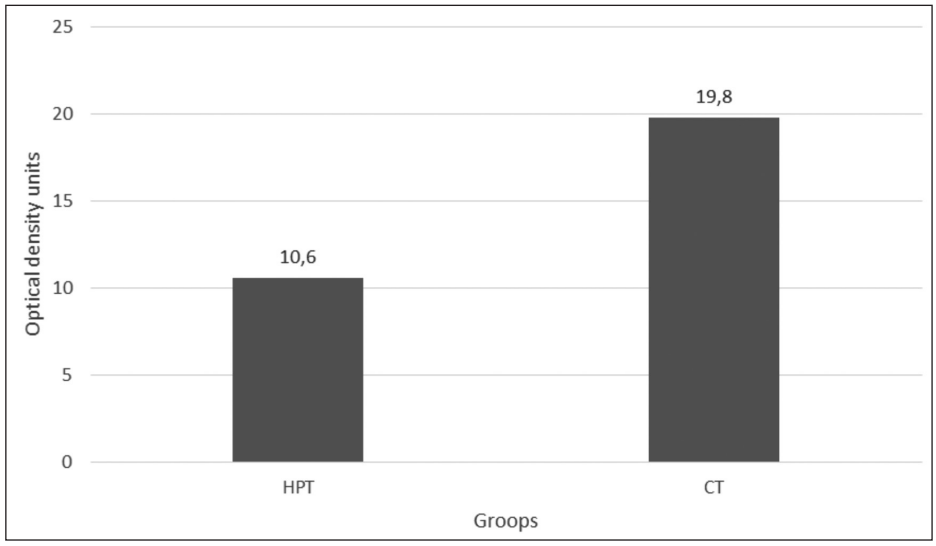

Fig. 1. The content of immune complexes in extracts from tonsils in case of HPT and CT.

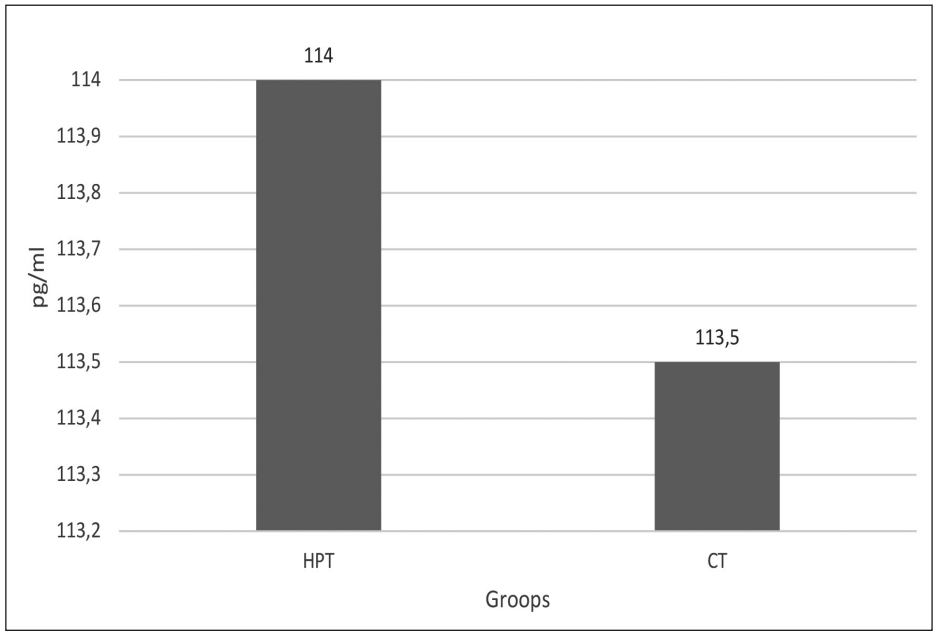

Fig. 2. The content of interleukin-4 in extracts from tonsils in case of HPT and CT.

form determination by immunoenzyme method (Hema reagents, RF).

In carrying out the research, the principles of bioethics and the legal requirements and requirements for conducting biomedical research were respected, namely: the Helsinki Declaration (2000), the Constitution (1996) and the Civil Code of Ukraine (2006), the Fundamentals of Ukrainian Health Law (1992), Guidelines for clinical investigations of the Ministry of Health of Ukraine No. 42-7.0: 2005 "Medicines. Appropriate clinical practice "(2005), Order of the Ministry of Health of Ukraine No. 66" On Approval of the Procedure for Conducting Clinical Trials of Medicinal Products and Examination of Materials for Clinical Trials and Model Regulations on the Ethics Commission "(2006), Law of Ukraine No. 3447-IV" On the Protection of Animals from ill-treatment "(2006), which is confirmed by the corresponding conclusion of the Ethical Committee of Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine (2015).

RESULTS

Data on the content of immune complexes are presented in Figure 1, which shows that the number of immune complexes in tonsillar tissues is twice lower in case of hypertrophy than in case of chronic tonsillitis.

Data on the content of interferons in extracts from tonsils in case of hypertrophy and chronic tonsillitis are presented in Table I.

Data on the content of interleukin-4 are presented in Figure 2, and the ratio of γ-interferon / interleukin-4 in Figure 3.

From the data in Figure 2 it follows that the levels of this cytokine in case of hypertrophy of palatine tonsils and chronic tonsillitis are about the same, and the ratio of γ-IFN and IL-4 is less in case of chronic tonsillitis (0.93) than in case of hypertrophy of palatine tonsils (1.21) (Figure 3). It indicates a greater tendency of the tonsillar tissue to form an immunopathological pro-allergic response in case of chronic tonsillitis [11].

Table I. The content of α and γ interferons in tissue extracts in case of HPT and CT.

Groups	α-IFN, pg/ml	γ-IFN, pg/ml
HPT (n=11)	41.0 (34-50)*	137.8 (91-190)**
CT (n=14)	50.6 (38-84)*	106.0 (59-140)**

Note: * - p>0.05, ** - p=0.065.

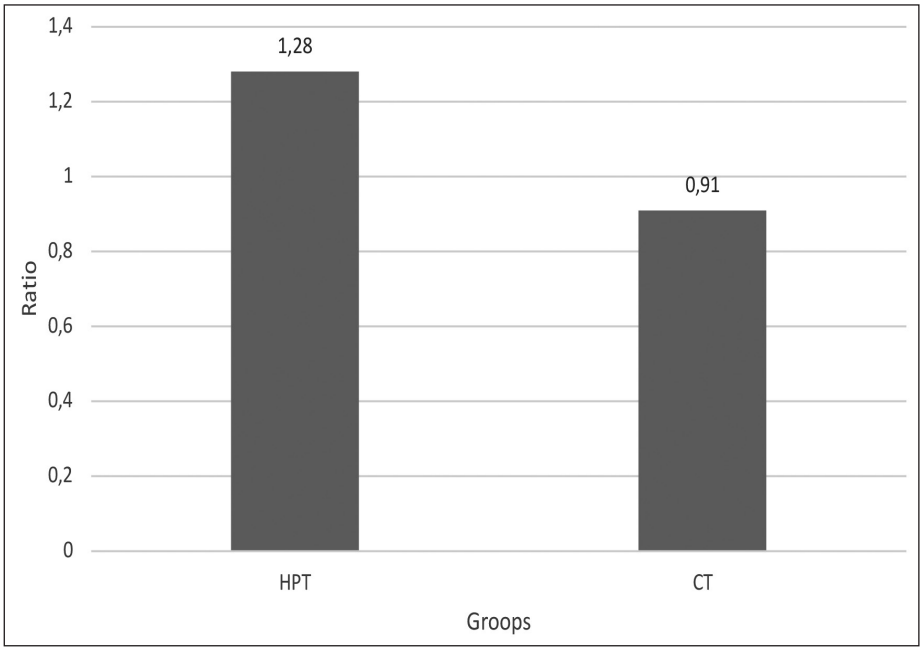

Fig. 3. The ratio of γ-interferon/interleukin-4 content in extracts from tonsils in case of HPT and CT.

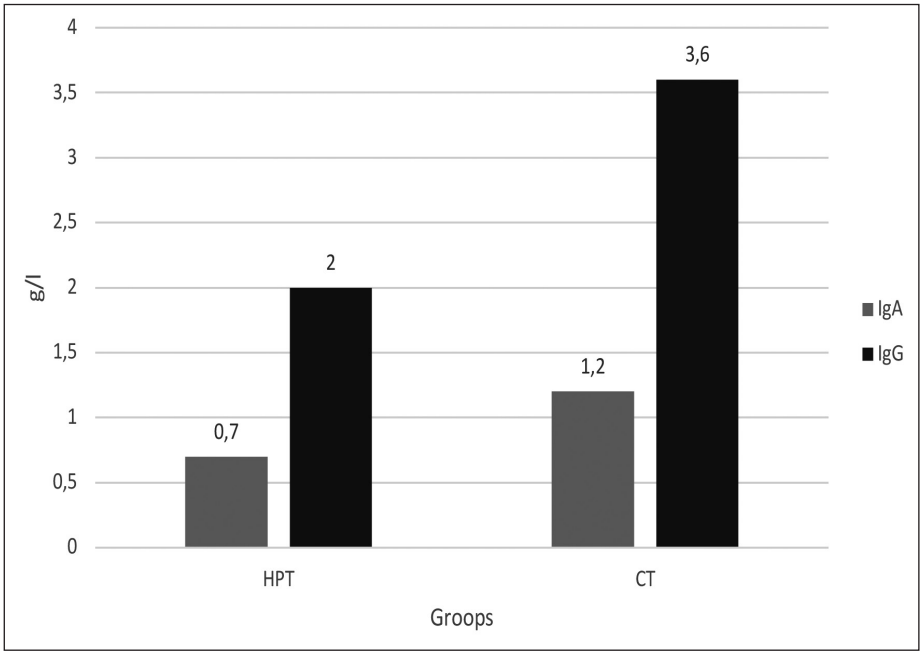

Fig. 4. IgA and IgG levels (g/l) in extracts from tonsils in case of hypertrophy and chronic tonsillitis.

From the data in Table II it follows that significant differences between the groups were only in relation to pro-inflammatory IL-1β, the content of which is significantly higher in case of chronic tonsillitis, which according to early research [12] directly indicates the presence of an inflammatory process in the tonsils of children.

IgA and IgG levels in extracts are presented in Figure 4. It can be seen that immunoglobulins A are contained in extracts from HPT and CT at about the same concentration with an unreliable prevalence in extracts from CT (HPT – 0.7 g/l and CT 1.2 g/l). IgG content was significantly higher in tissue extracts of patients with CT (2.0 g/l in case of HPT and 3.6 g/l in case of CT).

Table II. The content of interleukins 1β and 10 in tissue extracts from tonsils in case of hypertrophy of palatine tonsils and chronic tonsillitis.

Groups	IL-1β, pg/ml	IL-10, pg/ml
HPT (n=11)	136.0 (102-184)*	12.5 (9-20)**
CT (n=14)	195.8 (171-305)*	18.0 (9-24)**

Note: * - $p<0.05$, ** - $p=0.1$.

DISCUSSION

Our fin dings indicate that in tissue extracts from tonsils with CT obtained ad mass, there is a predominance of inflammation factors and potential sensitization, as well as the development of immunopathological reactions. The presence of inflammation is indicated by elevated levels of interleukin-1β, immunoglobulin G [13, 3]. High levels of interleukin-4 may indicate that both HPT and CT have a tendency to increase the activity of Th-2 cells, which means that hypertrophy and chronic tonsillitis in children can appear both in the form of sensitization to microbial and other antigens, and in the form of allergic inflammation, as evidenced in the study of cytokine levels in case of other inflammatory processes [11]. Based on the data presented and also taking into account the materials on chronic tonsillitis [2, 3, 4, 14] it may be considered appropriate to set the diagnosis of chronic tonsillitis, as recommended in the ICD-11 project.

CONCLUSIONS

1. The number of immune complexes in chronic tonsillitis is significantly ($p<0.05$) 1.86 times their number in tonsil hypertrophy.
2. The ratio of γ-IFN and IL-4 is less in case of chronic tonsillitis (0.93) than in case of hypertrophy of palatine tonsils (1.21).
3. The content of pro-inflammatory IL-1β is significantly higher ($p<0.05$) in case of chronic tonsillitis.
4. IgG content was significantly 1.8 times higher ($p <0.05$) in the tissue extracts of the tonsils of patients with CT than with HPT.
5. The content of α-IFN, γ-IFN and IgA of tonsils extracts with hypertrophy and chronic tonsillitis have no significant differences.

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ORIGINAL ARTICLE
PRACA ORYGINALNA

IMPROVEMENT OF STUDENTS' MORPHO-FUNCTIONAL DEVELOPMENT AND HEALTH IN THE PROCESS OF SPORT-ORIENTED PHYSICAL EDUCATION

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ABSTRACT

The aim is to investigate the influence of sambo wrestling classes on the level of morpho-functional development and health of students during their studying.

Materials and methods: The investigation was conducted in Zhytomyr National Agroecological University in 2017–2019. Ninety four students (51 male and 43 female) took part in the research. Two experimental and two control groups were formed: EG1 (n=25) and CG1 (n=26) involved male students, EG2 (n=21) and CG2 (n=22) – female students. The classes of EG were held according to the authors' methodology, the classes of CG – according to the current physical education program. The analysis of the indicators of weight, height, lungs capacity, handgrip test, heart rate, blood pressure, body mass index, life index, power index and Robinson's index was performed. The interrelation of students of EG and CG was defined according to the health levels at the beginning and at the end of the investigation.

Results: It is determined that sambo wrestling classes had a positive influence on the students' muscles system improvement, increased lung capacity, reduced body mass, improved cardiovascular system, and improved health.

Conclusions: The improvement of the indicators of morpho-functional development and health of students during sambo wrestling classes will have a positive influence on their physical working capacity and the efficiency of their studying and future professional activities.

KEY WORDS: physical education, morpho-functional development, health, sambo wrestling, student

Wiad Lek. 2020;73(1):161-168

INTRODUCTION

According to world standards, the system of reforming higher education in Ukraine forces the transformation of quantitative indicators of educational services into qualitative one that means the review of the content of higher education, its filling with the latest material and the implementation of modern learning technologies, including physical education [1, 2, 3]. According to many scholars [4, 5, 6, 7], physical education and sports should be considered in higher education institutions (HEI) as the basis for the professional health of future professionals, providing them with the competitiveness in the world market of professions. However, despite the improvement of the system of higher education in Ukraine, there is downtrend in health deterioration and physical fitness level of students [8, 9, 10, 11]. Besides, there is an urgent problem to increase students' interest in the quality of studying, to encourage them to systematically perform physical exercises and sports, and to form students' physical development and improvement needs for health and professional longevity [12, 13, 14, 15].

According to many scholars [16, 17, 18], one of the main reasons that prevent most students from engaging in physical health-improving and sports activities is the lack of a free choice of the type of motor activity. The studies of various scientists [18, 19, 20] determine that the number of students who would like to be engaged in sports sections while studying in HEI ranges from 50 to 80 % that proves the need to adjust the educational process of physical education considering interests, motivation and needs of students. Thus, the problem of increasing the motivation of students to perform physical exercises and sports and to comply with healthy lifestyle, increasing their physical fitness, morpho-functional development and health promotion can be solved through the implementation of sport-oriented classes in physical education, taking into account the students' free choice of the sport. In addition, it should be considered the popularity of the sports among students, the possibility of training and sports base of a HEI and the presence of specialists in sports in the teaching staff of the department of physical education.

Table 1. The dynamics of the weight and height indicators of EG and CG students during the experiment (X±m, n=94)

The stages of the experiment	Male students			Female students		
	EG1 (n=25)	CG1 (n=26)	p (EG1-CG1)	EG2 (n=21)	CG2 (n=22)	p (EG2-CG2)
			Height, sm			
1st term	175.9±1.12	176.1±1.04	>0.05	163.1±0.94	162.8±0.87	>0.05
2nd term	176.4±1.09	176.5±1.03	>0.05	163.5±0.92	163.2±0.85	>0.05
3rd term	176.6±1.08	176.8±1.01	>0.05	163.6±0.91	163.4±0.84	>0.05
4th term	176.9±1.06	177.2±0.98	>0.05	163.8±0.91	163.7±0.83	>0.05
p(1–4)	>0.05	>0.05		>0.05	>0.05	
			Weight, kg			
1st term	72.8±1.13	72.5±1.19	>0.05	58.1±0.85	57.9±0.79	>0.05
2nd term	72.1±1.15	73.1±1.16	>0.05	57.3±0.82	58.7±0.81	>0.05
3rd term	71.4±1.08	73.3±1.15	>0.05	56.8±0.76	59.6±0.80	<0.05
4th term	69.2±1.06	73.6±1.13	<0.01	56.1±0.71	60.4±0.82	<0.001
p(1–4)	<0.05	>0.05		<0.05	<0.05	
			Body mass index, kg/m²			
1st term	23.5±0.27	23.4±0.24	>0.05	21.8±0.30	21.8±0.28	>0.05
2nd term	23.2±0.25	23.3±0.23	>0.05	21.3±0.29	22.1±0.27	>0.05
3rd term	22.7±0.24	23.4±0.23	<0.05	21.1±0.28	22.4±0.27	<0.01
4th term	22.1±0.22	23.5±0.24	<0.001	20.8±0.26	22.5±0.28	<0.001
p(1–4)	<0.001	>0.05		<0.001	>0.05	

Notes: p(EG1–CG1) – significance of difference between the indicators of EG1 and CG1 due to the t-test;
p(EG2–CG2) – significance of difference between the indicators of EG2 and CG2 due to the t-test;
p(1–4) – significance of difference between the indicators of each group at the beginning and at the end of the experiment due to the t-test

The analysis of literature showed that a number of studies which consider the effectiveness of the priority use of different sports in the process of physical education in HEI, in particular various types of wrestling sports [21, 22, 23] have been carried out. At the same time, the least investigated is the issue of health promotion and improvement of morpho-functional development of both male and female students by the means of sambo wrestling.

THE AIM

The aim of the article is to investigate the influence of sambo wrestling classes on the level of morpho-functional development and health of students during studying at higher educational institutions.

MATERIALS AND METHODS

To achieve the aim of the investigation we conducted a pedagogical experiment in 2017–2019. Ninety four students (51 male and 43 female) of Zhytomyr National Agroecological University took part in the research. Two experimental and two control groups were formed: EG1 (n=25) and CG1 (n=26) involved male students (n=51), EG2 (n=21) and CG2 (n=22) – female students (n=43). The experimental and control groups included the students of the 1st year of study of the faculty of technology, aged 17–20, who had statistically equivalent indicators of physical fitness, morpho-functional development and health (p>0.05). The classes of EG1 and EG2 were held according to the authors' methodology by the means of sambo wrestling with the coaches for this type of wrestling sport, the classes of CG1 and CG2 – according to the current program of physical education at an agricultural HEI with the teachers of the department of physical education. The total number of hours spent on physical education per week for students of both groups was the same and equaled 4 hours. Monitoring of the level and dynamics of the indicators of morphological and functional development and health of students was carried out 4 times during the first and the second years of study (in 1st – 4th terms) – 4 stages of the investigation.

The analysis of the indicators of weight, height, lungs capacity, handgrip test, heart rate at rest, systolic and diastolic blood pressure, body mass index (the ratio of body weight to height), life index (the ratio of vital capacity to body weight), power index (the ratio of the wrist dynamometry to body weight), Robinson's index (a product of heart rate and systolic blood pressure) was performed. The interrelation of the students of EG and CG was defined according to the levels of health at the beginning and at the end of the investigation. The authenticity of the difference in the indicators of the students of EG and CG at the beginning and at the end of the investigation was defined due to the Student's test and the dynamics of the indicators during the research was analyzed.

Table 2. The dynamics of the physical development indicators of EG and CG students during the experiment (X±m, n=94)

The stages of the experiment	Male students			Female students		
	EG1 (n=25)	CG1 (n=26)	p (EG1-CG1)	EG2 (n=21)	CG2 (n=22)	p (EG2-CG2)
Lung capacity, ml						
1st term	4085.5±96.3	4105.8±93.9	>0.05	2873.5±89.1	2891.6±85.7	>0.05
2nd term	4192.6±95.1	4112.3±93.5	>0.05	2981.4±88.7	2907.3±85.4	>0.05
3rd term	4307.1±92.4	4117.4±92.8	>0.05	3092.2±88.5	2916.1±85.9	>0.05
4th term	4396.3±91.8	4122.1±92.5	<0.05	3206.7±88.3	2921.5±86.1	<0.05
p(1–4)	<0.05	>0.05		<0.05	>0.05	
Life index, ml/kg						
1st term	55.8±1.23	56.4±1.27	>0.05	49.4±1.37	49.8±1.42	>0.05
2nd term	58.2±1.28	56.2±1.26	>0.05	51.9±1.35	49.5±1.40	>0.05
3rd term	60.3±1.26	56.1±1.27	<0.05	54.3±1.33	49.1±1.41	<0.05
4th term	63.5±1.25	56.0±1.28	<0.001	56.8±1.31	48.4±1.43	<0.001
p(1–4)	<0.001	>0.05		<0.001	>0.05	
Handgrip test, kg						
1st term	38.6±0.79	39.1±0.72	>0.05	22.4±0.62	22.6±0.57	>0.05
2nd term	41.4±0.76	39.6±0.70	<0.05	26.1±0.57	22.8±0.58	<0.001
3rd term	44.6±0.75	40.5±0.71	<0.001	28.5±0.53	23.1±0.56	<0.001
4th term	47.7±0.70	40.9±0.69	<0.001	30.8±0.51	23.2±0.55	<0.001
p(1–4)	<0.001	>0.05		<0.001	>0.05	
Power index, %						
1st term	53.1±1.05	52.9±1.13	>0.05	38.5±1.16	39.1±1.18	>0.05
2nd term	57.4±1.01	54.1±1.12	<0.05	45.5±1.14	38.8±1.17	<0.001
3rd term	62.5±0.97	55.2±1.10	<0.001	50.1±1.13	38.8±1.18	<0.001
4th term	68.9±0.95	55.6±1.11	<0.001	54.8±1.10	38.5±1.19	<0.001
p(1–4)	<0.001	>0.05		<0.001	>0.05	

Notes: p(EG1–CG1) – significance of difference between the indicators of EG1 and CG1 due to the t-test;
p(EG2–CG2) – significance of difference between the indicators of EG2 and CG2 due to the t-test;
p(1–4) – significance of difference between the indicators of each group at the beginning and at the end of the experiment due to the t-test

The methods of investigation: theoretical analysis and generalization of scientific and methodological literature, pedagogic observation, questionnaire survey, biomedical methods, and methods of mathematical statistics.

RESULTS

In accordance with the normative documents regulating the organization of the educational process in physical education at HEI of Ukraine, the training sections of the department of physical education and the relevant training groups are completed in the first year of study at the beginning of the academic year taking into account the sports interests of students, their health state, physical fitness, sports qualification. Data necessary for the recruitment of the department's training sections can be obtained by questioning the students [2, 3]. The students who are interested in their sports skills improvement of this section were related to the training section of sport improvement of the department of physical education. The number and type of training groups is determined by the department of physical education taking into account material opportunities and staff [2, 4].

Having questioned students at the beginning of the 1st year of study in 2017, we determined that 29.8 % male students and 31.5 % female students were interested in sambo wrestling. Referring to the works of leading scientists [5, 7, 12, 22, 24, 25] and considering the results of personal researches [11, 26], we created the authors' methodology of the students' of HEI of Ukraine health and morpho-functional development improvement by the means of sambo wrestling. The main tasks of authors' methodology are determined to be increasing the interest and desire of students to take physical education lessons; improving morphological and functional development and improving student health. In order to verify the effectiveness of authors' methodology, we conducted a pedagogical experiment.

The analysis of the students' height showed no authentic difference in the indicators of EG and CG at the beginning

Table 3. The dynamics of the functional state indicators of EG and CG students during the experiment (X±m, n=94)

The stages of the experiment	Male students			Female students		
	EG1 (n=25)	CG1 (n=26)	p (EG1-CG1)	EG2 (n=21)	CG2 (n=22)	p (EG2-CG2)
Heart rate at rest, beats/min						
1st term	73.2±0.72	72.8±0.69	>0.05	70.1±0.73	69.9±0.77	>0.05
2nd term	72.9±0.70	72.7±0.68	>0.05	69.4±0.72	70.1±0.78	>0.05
3rd term	71.5±0.69	72.7±0.67	>0.05	68.1±0.71	70.2±0.79	>0.05
4th term	69.8±0.68	72.9±0.68	<0.01	67.2±0.69	70.0±0.78	<0.01
p(1–4)	<0.01	>0.05		<0.01	>0.05	
Systolic blood pressure, mmHg						
1st term	121.5±0.87	122.1±0.92	>0.05	116.6±0.13	116.5±0.85	>0.05
2nd term	121.2±0.86	121.9±0.91	>0.05	116.1±0.82	116.7±0.86	>0.05
3rd term	120.3±0.86	121.8±0.80	>0.05	115.7±0.81	116.5±0.86	>0.05
4th term	119.7±0.85	121.9±0.80	>0.05	115.3±0.81	116.6±0.85	>0.05
p(1–4)	>0.05	>0.05		>0.05	>0.05	
Diastolic blood pressure, mmHg						
1st term	74.2±0.76	73.9±0.81	>0.05	71.1±0.84	70.8±0.87	>0.05
2nd term	73.5±0.75	74.1±0.79	>0.05	70.7±0.82	71.1±0.88	>0.05
3rd term	73.3±0.74	74.0±0.78	>0.05	70.1±0.84	71.0±0.86	>0.05
4th term	72.9±0.73	74.2±0.80	>0.05	69.2±0.80	71.3±0.88	>0.05
p(1–4)	>0.05	>0.05		>0.05	>0.05	
Robinson's index, c.u.						
1st term	88.9±1.32	88.8±1.28	>0.05	81.7±1.39	81.4±1.35	>0.05
2nd term	88.3±1.31	88.6±1.28	>0.05	80.5±1.38	81.8±1.35	>0.05
3rd term	86.1±1.30	88.5±1.30	>0.05	78.9±1.36	81.8±1.36	>0.05
4th term	83.6±1.29	88.8±1.31	<0.01	77.5±1.35	81.6±1.34	<0.05
p(1–4)	<0.05	>0.05		<0.05	>0.05	

Notes: p(EG1–CG1) – significance of difference between the indicators of EG1 and CG1 due to the t-test;
p(EG2–CG2) – significance of difference between the indicators of EG2 and CG2 due to the t-test;
p(1–4) – significance of difference between the indicators of each group at the beginning and at the end of the experiment due to the t-test

of the experiment (p>0.05) (Table 1). A similar trend is observed at all other stages of the study. The students] height dynamics analysis also indicates that the indicators were increased during the experiment, but there was no significant difference in the initial and final data in both groups (p>0.05). It proves the lack of influence of both the classes according to the authors' methodology, and the classes according to the current physical education program on the height of male and female students.

The indicators of the body weight of EG and CG students do not have a significant difference at the beginning of the experiment (p>0.05) (Table 1). The body weight of EG students (both male and female) is lower than the one of the CG students at the 2nd and the 3rd stages of the experiment, but the difference is not authentic (p>0.05). At the last stage of the experiment, the EG students' body weight is lower authentically than the one of the CG students – the difference in the indicators of EG1 and CG1 is 4.4 kg (p<0.01); the difference in the indicators of EG2 and CG2 is 4.3 kg (p<0.001). The indicators of EG were decreased authentically during the experiment – the difference in body weight at the beginning and at the end of the experiment is 2.1 kg for EG1 and 2 kg for EG2 (p<0.05). The body weight of the students of CG has downtrend during the research: the body weight of the CG1 students is 1.1 kg higher at the 4th stage than at the beginning of the experiment (p>0.05), the CG2 students – 2.5 kg higher (p<0.05). It proves the efficiency of the authors' methodology – the sambo wrestling classes provide authentic body weight decrease of the EG students (both male and female). The analysis of body mass index showed no authentic difference in the indicators of EG and CG at the 1st and the 2nd stages of the experiment (p>0.05) (Table 1). At the 3rd and the 4th stages, the students of EG were noted to have significantly higher indicators than CG: the indicators of male students were for 0.7 kg/m^2 higher at the 3rd stage (p<0.05), and for 1.4 kg/m^2 at the 4th stage (p<0.001); the indicators of female students were for 1.3 kg/m^2 higher at the 3rd stage (p<0.01), and for 1.7 kg/m^2

Table 4. The interrelation of EG and CG students concerning the health level during the experiment (X±m, n=94, %)

Health levels	Stages of the experiment			
	The beginning	The end	The beginning	The end
Male students				
	EG1 (n=25)		CG1 (n=26)	
Low	64.0	8.0	65.4	57.7
Below the middle	20.0	16.0	15.4	19.2
Middle	12.0	52.0	11.5	15.4
Above the middle	4.0	24.0	7.7	7.7
High	-	-	-	-
Female students				
	EG2 (n=21)		CG2 (n=22)	
Low	57.1	14.4	59.0	50.0
Below the middle	23.8	19.1	18.2	22.7
Middle	19.1	52.4	18.2	22.7
Above the middle	-	19.1	4.6	4.6
High	-	-	-	-

at the 4th stage ($p<0.001$). The dynamics of the students' body mass index is characterized by the EG students' uptrend and CG students' downtrend of the indicator. Thus, the final indicator of the male students of EG1 is for 1.4 kg/m^2 better than the initial one authentically ($p<0.001$), the final indicator of the female students of EG2 – for 1 kg/m^2 ($p<0.05$). The body mass index of CG students was not changed authentically during the experiment ($p>0.05$).

The examination of lung capacity indicates that the indicators of EG and CG students are not significantly different at the 1st – 3rd stages of the experiment ($p>0.05$) (Table 2). At the 4th stage of the experiment the lung capacity of EG1 is for 274.2 ml better than the one of CG1 authentically ($p<0.05$), and the indicator of EG2 is for 285.2 ml better than the one of CG2 authentically ($p<0.05$). The analysis of the lung capacity dynamics showed that the indicators of EG1 and EG2 were for 310.8 ml and 333.2 ml improved respectively during the experiment authentically ($p<0.05$), and the indicators of CG were not changed authentically ($p>0.05$). The investigation of life index proves that the indicators of EG and CG are the same authentically at the 1st and the 2nd stages of the experiment ($p>0.05$). The life index of EG1 is for 4.2 ml/kg better than the one of CG1 authentically ($p<0.05$) at the 3rd stage and 7.5 ml/kg better at the 4th stage ($p<0.001$). The difference in the indicators of EG2 and CG2 is 5.2 ml/kg at the 3rd stage ($p<0.05$) and 8.4 ml/kg at the 4th stage ($p<0.001$). The life index dynamics of the students of EG has a positive character – the difference in the indicators at the beginning and at the end of the experiment is 7.7 ml/kg for EG1 and 7.4 ml/kg for EG2 ($p<0.001$) that shows the improvement of the functional abilities of the students' respiratory system (both male and female) during the sambo wrestling classes. The life index indicators of CG were worsened but not changed authentically ($p>0.05$) (Table 2).

The handgrip test analysis shows that the indicators of EG and CG students do not differ authentically just at the 1st stage of the investigation ($p>0.05$). The indicators of the arm muscles power of EG1 are for 1.8 kg better than the ones of CG1 at the 2nd stage, for 4.1 kg at the 3rd stage, for 6.8 kg at the 4th stage authentically ($p<0.05$–0.001). The indicators of handgrip test of EG2 are for 3.3 kg better than the ones of CG2 at the 2nd stage, for 5.4 kg at the 3rd stage, for 7.6 kg at the 4th stage authentically ($p<0.001$) (Table 2). The power characteristics of EG students are being improved during all stages of the experiment – they are for 9.1 kg better for EG1 and for 8.4 kg better for EG2 at the end of the experiment than at the beginning of the experiment authentically ($p<0.001$) that proves the efficiency of the authors' methodology. The indicators of the handgrip test of CG were not changed authentically during the pedagogical experiment ($p>0.05$).

The examination of power index proves that the indicators of EG and CG are not authentically different at the beginning of the experiment ($p>0.05$). The next stages shows the significant influence of the classes according to the authors' methodology: the difference in the indicators of EG1 and CG1 is 3.3 % at the 2nd stage ($p<0.05$), 7.5 % at the 3rd stage ($p<0.001$), 13.3 % at the 4th stage ($p<0.001$). The difference in the indicators of power index of the female students of EG2 and CG2 is also authentic at the 2nd – 4th stages ($p<0.001$) and it equals 6.7 %, 11.3 % and 16.3 % respectively (Table 2).

The analysis of heart rate showed that the indicators do not differ authentically at the 1st – 3rd stages of the investigation ($p>0.05$). The difference in the indicators of EG1 and CG1 is 3.1 beats/min, EG2 and CG2 – 2.8 beats/min at the end of the research ($p<0.01$) (Table 3). The dynamics of heart rate of EG is characterized by authentic decrease of heart rate during the experiment – the indicators of the 4th stage are for 3.4 beats/min and 2.8 beats/min lower for EG1 and EG2 respectively than the ones of the 1st stage authentically ($p<0.01$). The heart rate of the students of

CG was not changed authentically during the research (p>0.05). The analysis of blood pressure showed that the indicators of EG and CG were not authentically different at all stages of investigation (p>0.05) (Table 3). The dynamics of the blood pressure indicators of EG have downtrend that indicates the improvement of the cardiovascular system activity of the students who were training according to the authors' methodology. The difference in the initial and the final indicators of the systolic blood pressure of EG1 is 1.8 mmHg, EG2 – 1.3 mmHg (p>0.05); diastolic blood pressure of EG1 – 1.3 mmHg, EG2 – 1.9 mmHg (p>0.05). The blood pressure indicators of CG students stayed almost not changed during the experiment (p>0.05).

The analysis of the students' Robinson's index showed no authentic difference in the indicators of EG and CG during the 1st – 3rd stages of investigation (p>0.05) (Table 3). The Robinson's index of EG1 is for 5.2 c.u. better than the one of CG1 authentically and the indicators of EG2 are for 4.1 c.u. better than the ones of CG2 authentically (p<0.05) at the 4th stage of the investigation. The examination of the students' Robinson's index dynamics proves that the classes according to the authors' metodology improve the cardiovascular system activity of the students of EG – Robinson's index of EG1 and EG 2 was authentically for 5.3 c.u. and for 4.2 c.u. improved respectively (p<0.05). The Robinson's index indicators of CG1 and CG2 were not authentically different at the beginning and at the end of the research (p>0.05). It emphasizes the positive influence of the classes according to the authors' methodology on the indicators of the functional abilities of the EG students` cardiovascular system.

The analysis of the students' interrelation concerning the levels of their health showed that at the beginning of the experiment the majority of the students (both male and female) had low and below the middle health levels: the students of EG1 – 84 %, CG1 – 80.8 %, EG2 – 80.9 %, CG2 – 77.2 % (Table 4).

At the end of the experiment, 52 % male students of EG1 had a middle health level; 24 % – above the middle; 52.4 % female students of EG2 were determined to have a middle level of health, 19.1 % – above the middle. 76.9 % male students of CG1 and 72.7 % female students of CG2 were defined to have a low and below the middle health levels at the end of the research.

DISCUSSION

The scientists [1, 8, 12, 27] mention that the studentsə unsatisfactory health level is related to the state of health of the population of Ukraine. The investigations [7, 9, 10, 28] prove that the average expected life duration of men was for 2.4 years decreased, of women – for 0.9 years decreased. The difference in life expectancy in Ukraine and in Western Europe is 12.8 years for the male population, and 7.8 years for the female population. Cardiovascular pathology is increased for 1.9 times in average, bronchial asthma for 35.2 %, and diabetes for 10.1 %. It is primarily conditioned by the way of life of modern youth and, in particular, the low level of their motor activity. It confirms the low level of morpho-functional development and health of students. Besides, the specialists [5, 6, 13, 14] claim that the insufficient health level of students is also caused by a decrease in their interest in physical education and sports. The students' low motivation is reflected in the attendance of scheduled physical education classes. The most of students attend physical education classes only to fulfill the requirements of the curriculum to pass an exam. According to the data of many scientists [16, 17, 18], one of the perspective directions for solving these problems is the implementation of the sport-oriented physical education classes, taking into account the students' free choice of sport.

The results of our research show that the classes in physical education according to the authors' methodology influenced the strengthening of the body of students positively. Thus, systematic sambo wrestling classes influenced the improvement of the muscular system of both male and female students, the increase of the lung capacity, the decrease of their body weight, and the improvement of cardiovascular system performance concerning the indicators of heart rate and blood pressure. The improvement of these indicators will influence the physical capacity of the students, their state of health, well-being and the effectiveness of educational activities at senior courses of study and future professional activity positively.

CONCLUSIONS

1. Both male and female students of EG are determined to have authentically better (p<0.05–0.001) indicators of morpho-functional development at the end of the experiment, in contrast to the students of CG: body weight – for 4.4 and 4.3 kg; lung capacity – for 274.2 and 285.2 ml; handgrip test (wrist dynamometry) – for 6.8 and 7.6 kg; heart rate – for 3.1 and 2.8 beats/min; body mass index – for 1.4 and 1.7 kg/m^2; life index – for 7.5 and 8.4 ml/kg; power index – for 13.3 and 16.3 %; Robinson's index – for 5.2 and 4.1 c.u.
2. It is defined that the number of the male students of EG who have middle and above the middle health level was increased from 16 to 76 %, the female students of EG – from 19.1 to 81.5 % at the end of the investigation that proves a positive influence of the authors' methodology.
3. The implementation of the authors' methodology ensured the engagement of students in systematic exercises that allowed improving their level of morpho-functional development and health. It will improve the effectiveness of their studying and future professional activities.

The prospects of future investigations involve the research of the studying the motivation of students of higher educational institutions of different specialties to healthy life stile.

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REVIEW ARTICLE
PRACA POGLĄDOWA

ANALYSIS OF MEDICAL SERVICES FINANCIAL EFFICIENCY IN THE SECONDARY CARE INSTITUTIONS OF SUMY REGION

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ABSTRACT

The aim: The aim of the study is to analyze financial indicators and assess the effectiveness of financing secondary medical care enterprises during the transition period in the context of remuneration to doctors.

Materials and methods: To perform the analysis, the financial characteristics of 26 medical facilities have been collected and processed by means of involving the biblio-semantic method, data extract and content analysis.

Conclusions: Presents a comparative analysis of labour payment expenses to doctors of cardiology, obstetric and gynecological and surgical departments of medical facilities. The predicted labour payment expenses for the number of services provided have been calculated. Besides, the article makes it clear that the allocation of funds according to bed-day loads and the number of medical services rendered is unsustainable and does not correspond to the real load on the actual position.

KEY WORDS: financing, costs, remuneration, medical institutions

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INTRODUCTION

PROBLEM FORMULATION

The key to the development and prosperity of each country is healthy citizens. Accordingly, healthcare is a priority for improvement and development. Today, the healthcare system is in the process of reforming. Tariff setting system for rendered services which had existed before the reform started and which to some extent retains its function in the transition period did not take into account many factors that eventually affect the quality level of medical services. The financial component of the health care system is divided into funding salaries, administrative expenses, procurement of pharmaceuticals, etc. The financing model proposed by power-makers aims at introducing a state guaranteed package of medical care. It is based on the main principle of changes which consists in the shift from funding cost estimates of medical institutions to payment for the result [1]. Although the implementation of new legislative norms and work systems has been under way not long so far, and it mainly refers to primary health care, currently, we can observe both positive changes and problematic issues that require elaboration and identification of the ways to solve them. Currently, the issue that needs to be thoroughly studied is secondary healthcare financing, that will enable efficient distribution and employment of the funds rendered. At the same time, secondary medical institutions are supported by medical subvention funds from the state and local budgets. There are a lot of health care facilities where funds are spent only on their passive maintenance, or use of funds does not meet improving the quality of medical care and the real workload. Therefore, in order to determine the effectiveness of funding, it is necessary to undertake a thorough cost analysis using the example of remuneration for health professionals.

PUBLICATIONS ANALYSIS

The issue of medical system and health care reform in Ukraine is being explored by scientists from various fields of science, since these areas have multiple components that are worth analyzing, studying and processing. Publications of various scholars both cover new approaches to work in the face of changes and actively analyze the still operating system. Thus, S.O. Kushnir is considering financial security issues with the problems of financial support in the process of reform, focusing on the level of wages and staffing of medical institutions [2].

I.S. Muzyka when considering the implementation of medical reform on the example of the Carpathian region, Ukraine, concludes that the existing financing mechanism does not fully allow for the specifics and features of a particular region. [3].

I.V. Yaremchuk pays attention to the public internal control over financial flows in the context of healthcare reform. Having conducted the cost analisis for several years, he concluded that, despite increased costs, the overall situation in medicine has not improved significantly [4].

L.V. Hryniv and T.V. Pushik dedicate their research to the issues of medical facility management in the context of medical reform, and conclude that quality services are guaranteed by the availability of skilled and motivated staff,

Table 1. Labour costs for one treated patient per year, UAN

The name of a Medical Facility	Cash labor costs (KEKV 2110) for 2018, thousand UAH	Bed capacity as of 01.01.2019	Number of patients treated for 2018	Labour costs per 1 patient, thousand UAH	Labour costs outlook per 1 patient, UAH, in terms of the reform	Percentage of patients treated of the total number by districts
Bilopillia Central District Hospital (Bilopillia CDH)	10567	200	6 962	52,84	4859,80	4,02
Buryn CDH	6910	105	3 764	65,81	11196,19	2,17
Velyka Pysarivka CDH	5252	100	2992	52,52	11240,72	1,73
Krasnopillia CDH	6284	125	2978	50,27	10810,17	1,72
Krolevets CDH	10892,9	160	5013	68,08	8696,75	2,90
Lebedyn CDH	11277	162	6264	69,61	7116,36	3,62
Lypova Dolyna CDH	4503	94	2592	47,90	11835,05	1,50
Nedryhailiv CDH	6975	100	3713	69,75	12029,58	2,15
Putyvl CDH	9194	135	3893	68,10	11202,56	2,25
Seredyno-Buda CDH	4957	94	2528	52,73	13358,11	1,46
MNO "Sumy CDCH" of SDC, SR (Municipal Non-Commercial Organization "Sumy Central District Clinical Hospital" of Sumy District Council, Sumy Region	11062	215	7291	51,45	4518,97	4,21
Yampil CDH	4737	91	3050	52,05	10929,32	1,76
MF (Municipal Facility) "Sumy City Clinical Hospital № 1"	25485	360	12315	70,79	3681,11	7,12
MNO "Clinical Hospital № 4" of SCC (Sumy City Council)	8745	130	6040	67,27	7131,99	3,49
MF (Municipal Facility) "Sumy City Clinical Hospital № 5"	22557	330	10928	68,35	4005,51	6,31
MNO "The St Zinaida Children's Hospital" of SCC	20331	295	10729	68,92	4113,47	6,20
MNO "The Blessed Virgin Mary Clinical Maternity Hospital" of SCC	11552	165	4566	70,01	9819,03	2,64
Konotop CDH	33392	521	19718	64,09	2081,48	11,39
MNO of KCC "Konotop City Hospital"	6132	110	4308	55,75	8286,38	2,49
Romny CDH	23892	350	12837	68,26	3405,27	7,42
Shostka CDH	22860	340	11418	67,24	3770,84	6,60
MNO "Shostka Children's Hospital" of Shostka City Council	4496	65	2090	69,17	21193,26	1,21
Okhtyrka CDH	17642	326	12462	54,12	2780,82	7,20
Hlukhiv CDH	19529,75	265	8477	73,70	5567,24	4,90
Trostianets City Hospital	11660	170	6145	68,59	7147,58	3,55

Note: Compiled by the authors according to the data from [14].

and applying a value-based healthcare system at the micro level allows to achieve cost savings, to improve the quality of medical services, and at the state level – to increase health care efficiency [5].

V.A. Smiianov developed and implemented a model of internal audit at the institution of health care. This mechanism helps to optimize financial, organizational and treatment processes in hospitals, leading to improved quality of care. [6].

T. Zhelyuk talks about the scientific and applied aspects of work, which should be aimed at effectiveness capable of contributing to the development of competitiveness at medical institutions. For this purpose, it is required to have a resource

Table 2. Activity analysis of inpatient obstetric and gynecological departments per one actual permanent positions, UAN

The name of a Medical Facility	Actually held positions	Number of patients treated for 2018	Average monthly labour costs in terms of actually held position, UAH	Average annual labour costs in terms of actually held position, UAH	Cash labor costs (KEKV 2110) for 2018, thousand UAH	Number of patients treated per 1 actually held position	Labour costs per 1 patient treated, UAH, for actually held position	Bed capacity	Labor costs outlook per 1 bed, UAH	Projected labor costs for actually treated patients, UAH, per one established position
Bilopillia CRH	3,75	672	10 602	127 224	477090	179,20	709,96	20	11,01	66304,00
Buryn CDH	1,5	561	7161	85 932	128898	374,00	229,76	15	9,89	138380,00
Velyka Pysarivka CDH	1,25	674	27878	334 536	418170	539,20	620,43	18	9,88	199504,00
Krasnopillia CDH	1	261	9850	118 200	118200	261,00	452,87	15	21,26	96570,00
Krolevets CDH	5,5	630	6609	79 308	436194	114,55	692,37	18	10,57	42381,82
Lebedyn CDH	5	683	9288	111 456	557280	136,60	815,93	14	7,58	50542,00
Lypova Dolyna CDH	1	247	16175	194 100	194100	247,00	785,83	15	22,47	91390,00
Putyvl CDH	3,25	642	24079	288 948	939081	197,54	1462,74	25	14,41	73089,23
Sumy CDCH	1,5	773	8817	105 804	158706	515,33	205,31	20	9,57	190673,33
Yampil CDH	2	683	7017	84 204	168408	341,50	246,57	16	8,67	126355,00
MF "Sumy City Clinical Hospital № 1"	6,5	1632	6560	78 720	511680	251,08	313,53	40	9,07	92898,46
MF "Sumy City Clinical Hospital № 5"	9	1474	5404	64 848	583632	163,78	395,95	30	7,53	60597,78
MNO "The Blessed Virgin Mary Clinical Maternity Hospital" of SCC	208,5	4566	4598	55 176	11504196	21,90	2519,53	165	13,37	8102,73
Konotop CDH	14	2188	16995	203 940	2855160	156,29	1304,92	65	10,99	57825,71
Romny CDH	11,5	2034	14912	178 944	2057856	176,87	1011,73	50	9,10	65441,74
Shostka CDH	20,25	1756	11826	141 912	2873718	86,72	1636,51	60	12,64	32084,94
Okhtyrka CDH	10,75	1118	5881	70 572	758649	104,00	678,58	33	10,92	38480,00
Hlukhiv CDH	7,5	1687	12738,6	152 863	1146474	224,93	679,59	38	8,33	83225,33
Trostianets City Hospital	6	861	10003	120 036	720216	143,50	836,49	25	10,74	53095,00

Note: Compiled by the authors according to the data from [14].

base for functional activity, to strengthen the internal factors of competitiveness, such as medical efficiency, that is, the ability to meet the needs of patients by providing a full range of high-quality medical services and administrative effectiveness, including the effectiveness of administrative, financial, personnel, information and communication management [7].

S. Knysh considers the problems of medical reform from the point of view of legal regulation and speaks about the prevention of corruption in the management process. In his work, he says that corruption largely depends on the level of material security of medical professionals themselves. Adequate cash security together with the increased legal liability significantly facilitate the prevention of corruption offenses. The scientist emphasizes the main problem consisting in the fact that it is incorrect to require transparency and honesty in work whereas financing does not correspond to the complexity of the acquired profession. Medical reform is aimed at taking measures to remedy this situation, in particular, by improving the working conditions of health workers [8].

THE AIM

The aim of the study is to analyze financial indicators and assess the effectiveness of financing secondary medical care enterprises during the transition period in the context of remuneration to doctors.

Table 3. Labour costs for childbirth in a maternity ward per one actual established (permanent) positions, UAN

The name of a Medical Facility	Actually held positions	Cash labor costs (KEKV 2110) for 2018, thousand UAH	Number of births (deliveries) for 2018	Number of patients treated for 2018	Bed capacity	Labour costs per 1 patient, thousand UAH	Number of births per 1 actually held post	Labour costs outlook per 1 patient, UAH, in terms of the reform	Projected labor costs for actually treated patients, UAH, per one established position
Velyka Pysarivka CDH	0,75	750	33	37	8	20,27	44,00	80,00	16280,00
Putyvl CDH	1,5	1144	85	205	10	5,58	56,67	18,05	20966,67
Yampil CDH	1	561	73	285	6	1,97	73,00	7,79	27010,00
MNO "The Blessed Virgin Mary Clinical Maternity Hospital" of SCC	61	3366	1854	1860	75	1,81	30,39	14,92	11245,57
Konotop CDH	12	3484	695	1062	35	3,28	57,92	12,19	21429,17
Romny CDH	9,75	3086	404	1130	30	2,73	41,44	9,82	15331,28
Shostka CDH	16,25	4373	459	617	30	7,09	28,25	17,99	10451,08
Hlukhiv CDH	3,25	2022,8	362	952	19	2,12	111,38	7,38	41212,31

Note: Compiled by the authors according to the data from [14]

MATERIALS AND METHODS

In order to assess cost-effectiveness of medical services, we have analyzed the financial performance of inpatient departments of Sumy region's medical clinics rendering secondary medical care. These figures concern payroll costs to physicians. In total, 26 institutions are subordinated to the healthcare department of Sumy Regional State Administration.

Our computations are based on the following criteria: cash labour costs, average monthly labour costs per an actually held position, the number of patients treated, as well as bed capacity. The study was conducted with involving the bibliosemantic method, content analysis and data extract.

REVIEW AND DISCUSSION

In Ukraine, medical reform started with the adoption of the Laws "On State Financial Guarantees of Medical Services for the Population" of October 19, 2017, which entered into force on January 30, 2018, and "On Amendments to Some Legislative Acts of Ukraine on Improving Legislation on the Activities of Health Care Institutions" dated 06.04.2017, which came into force on 06.11.2017 [9]. This law provides for a change in the legal status of healthcare institutions to municipal non-commercial organizations (non-profit utility companies) that will enable to independently manage administrative, financial and business activities. The cardinal change in the reform is the transition from the completely state-subsidized medical institutions to public funding of particular health care services. Accordingly, the National Health Service of Ukraine has been established. It is a governing body that pays money to medical facilities for their services provided. Under the Medical Guarantee Program, citizens are ensured to have full payment at the expense of the state budget [10]. According to this law, a uniform tariff for medical service payment has been set. It may sustain modifications in compliance with coefficients. Change in funding system began with primary health care. An important component of the reform is the remuneration to health workers. However, in the transition period, secondary healthcare funding is still being carried out by the current system (at the expense of local budgets) [11].

Changes to the secondary health facilities are gradually entering into force. The key point of this reform is the modification of healthcare financing system, which consists in channeling funds for the services rendered by a healthcare facility to a particular patient. To calculate the cost of a service, the following indicators must be taken into account: the total budget allotment for the healthcare system, the cost of a particular service, and the need for a given service [12]. As of today, hospital financing is calculated on the basis of the number of people living on the territory accountable to a particular healthcare facility per inhabitant. The amount of financing for hospital maintenance on the state and local budgets is determined by the established economic norms and standards, based on bed capacity by a specific disease profile. It is according to those standards that the required number of doctors, mid-level and junior medical personnel, salary funds, costs on medications, nutrition, etc. are identified [13]. The amount of payment is formed on the basis of official salaries, which are approved by the Cabinet of Ministers of Ukraine. That is, healthcare workers are paid for the hours worked, but not for the volume of services rendered. This approach results in the lack of motivation in for healthcare employees to improve the quality of care and care. The existing load on hospital beds does not take into account the practical need for their number and duration of inpatient treatment without regard to the profile of hospital department, where in order to maintain the funding level, the unreasonable bed capacity is available.

Table 4. Activity analysis of inpatient surgical departments per one actually held established positions, UAN

The name of a medical facility	Actually held positions	Number of surgeries for 2018 without regard to skin and subcutaneous layer intervention	Average monthly labour costs in terms of actually held position, UAH	Average annual labour costs in terms of actually held position, UAH	Number of surgeries per 1 actually held post	Labour costs for 1 surgery, UAH, per actually held post	Bed capacity	Labour costs outlook per 1 bed, UAH	Projected labor costs for actually treated patients, UAH, per one established position
Bilopillia CDH	5,5	135	6 829	81 948	24,55	3338,62	25	68,52	9081,82
Buryn CDH	2	205	9129	109 548	102,50	1068,76	25	45,12	37925,00
Velyka Pysarivka CDH	1	150	16933	203 196	150,00	1354,64	22	54,27	55500,00
Krasnopillia CDH	1,5	73	13928	167 136	48,67	3434,30	15	76,03	18006,67
Krolevets CDH	6,5	188	8559	102 708	28,92	3551,07	45	88,56	10701,54
Lebedyn CDH	6,5	196	8149	97 788	30,15	3242,97	26	49,08	11156,92
Lypova Dolyna CDH	2,5	77	13331	159 972	30,80	5193,90	19	91,30	11396,00
Nedryhailiv CDH	4	178	12675	152 100	44,50	3417,98	31	64,44	16465,00
Putyvl CDH	4,75	290	12079	144 948	61,05	2374,15	30	38,28	22589,47
Seredyno-Buda CDH	4,25	142	9673	116 076	33,41	3474,11	30	78,17	12362,35
MNO "Sumy CDCH" of SDC, SR	3,5	589	6943	83 316	168,29	495,09	35	21,99	62265,71
Yampil CDH	2,5	116	9870	118 440	46,40	2552,59	25	79,74	17168,00
MF "Sumy City Clinical Hospital № 1"	9,75	640	8179	98 148	65,64	1495,22	40	23,13	24287,18
MF "Sumy City Clinical Hospital № 5"	18,25	937	18482	221 784	51,34	4319,70	110	43,44	18996,71
Konotop CDH	13	738	7372	88 464	56,77	1558,31	70	35,09	21004,62
MNO of KCC "Konotop City Hospital"	4	97	10373	124 476	24,25	5133,03	45	171,65	8972,50
Romny CDH	14	761	8524	102 288	54,36	1881,78	47	22,85	20112,14
Shostka CDH	12	664	7074	84 888	55,33	1534,12	50	27,86	20473,33
Okhtyrka CDH	9,5	456	8680	104 160	48,00	2170,00	55	44,63	17760,00
Hlukhiv CDH	6,75	298	8244,4	98 933	44,15	2240,93	48	59,60	16334,81
Trostianets City Hospital	5,5	202	12289	147 468	36,73	4015,22	30	54,95	13589,09

Note: Compiled by the authors according to the data from [14].

Labor costs per one person treated at secondary healthcare hospitals for 2018 presented in Table 1 on average have the following characteristics:

Table 1 clearly shows that bed capacity in hospitals has significant differences, as this figure is mainly calculated in accordance with the average annual population in regions. However, labour costs per patient are different. For example, comparing two medical establishments – Bilopillia Central District Hospital, which has 200 beds, and the number of treated is 6962 people, and Nedryhailiv Central District Hospital, where 100 beds and 3713 treated, the salary costs are 52,84 thousand UAN per year and 69.75 thousand UAN per year respectively. We can see that, compared to the Nedryhailiv CDH, the Bilopillia CDH has twice the load, but receives 24.24% less labourl costs. This discrepancy exists in other institutions.

A significant element of the Medical Guarantee Program will be medical services related to childbirth, treatment

Table 5. Activity analysis of inpatient cardiac departments per one actually held established position, UAN

The name of a medical facility	Actually held positions	Number of patients treated for 2018	Average monthly labour costs in terms of actually held position, UAH	Average annual labour costs in terms of actually held position, UAH	Cash labor costs (KEKV 2110) for 2018, thousand UAH	Number of patients treated per 1 actually held position	Labour costs per 1 patient treated, UAH, for actually held position	Bed capacity	Forecast	Projected labor costs for actually treated patients, UAH, per one established position
Lebedyn CDH	2,25	1294	6596	79152	178	575,11	137,63	33	9,44	212791,11
MNO "Sumy CDCH" of SDC, SR	3	929	4811	57732	173	309,67	186,43	30	11,95	114576,67
MF 'Sumy City Clinical Hospital № 1'	5	847	5143	61716	309	169,40	364,32	30	13,11	62678,00
MF "Sumy City Clinical Hospital № 5"	2,25	166	4044	48528	109	73,78	657,76		0,00	27297,78
Konotop CDH	3	1592	6250	75000	225	530,67	141,33	45	10,46	196346,67
Romny CDH	2	975	6496	77952	156	487,50	159,90	30	11,38	180375,00
Shostka CDH	2	795	6368	76416	153	397,50	192,24	25	11,64	147075,00

Note: Compiled by the authors according to the data from [14].

of cardiovascular diseases, screening for early detection of cancer, etc. Taking the example of obstetric and gynecological, cardiac, and surgical wards we can analyze the pre-reform payments and predict the total costs that healthcare establishments will be able to receive after reforming the financial relationships. The data are presented in Table 2 and Table 3.

Table 2 says that the number of patients per 1 actually held position makes a big difference, and labour costs do not match the actual workload. For example, the cost of remuneration per 1 patient treated in Bilopillia CDH is UAH 709.96, and the number of patients treated per 1 actually held position is 179.2 people. At that time, in Buryn CDH labor costs per 1 patient treated is UAH 229.76, and the number of patients treated per 1 actually held position is 374 people. The Shostka CDH has completely inappropriate figures – the cost of remuneration per 1 patient treated is 1636.51 UAH, while the number of patients treated per 1 actually held position is only 86.72 people.

We also analyzed those facilities that provide only obstetric services with regard to delivery costs. There are only 8 of them in Sumy region. Table 3 covers the available and projected indicators.

As we can see, there exists inconsistency and imbalance as to the load on maternity wards within district hospitals of the region. In order to optimize hospital departments and the rate of expenditure, as well as to increase the level of qualified assistance for childbirth, the minimum safe load will be determined. It is considered that 200 childbirths per year for one ward is insufficient for physicians to maintain the required level of qualification, and as the WHO has estimated, the minimum required number of childbirths should be 500 in order to be safe [15]. Table 3 shows that two of eight medical facilities departments have got more than 500 childbirths. But three of them do not exceed even 100 childbirths but labor costs are higher than in those departments which have got more than 500 cases.

A similar discrepancy situation is observed in surgery and cardiology departments, which is presented in Table 4 and Table 5.

Also remarcable is the situation with bed capacity. Having an equal load on the bed fund (Bilopillia, Buryn and Yampil CDHs), there is a big difference between the actually held positions and the number of surgeries fulfilled, and again we can see the fact that the workload does not match the labour costs.

We can conclude that the load on one permanent post at remuneration of labor in different hospitals within the same type of medical care does not correspond to the number and quality of services provided. It is obvious that labour costs are insufficient. It means if there are more patients treated then there is less expenditure on wages. It causes the absence of motivation that results in quality of health services.

CONCLUSIONS

Analyzing projected labor costs for actually treated patients per one established position it is necessary to mention that they can become quite sufficient and would create competitive atmosphere in healthcare industry.

The reform of the health care system is primarily aimed at improving the quality of healthcare support. The introduction of secondary healthcare reform, which is gradually extending, will contribute to a significant redistribution of financial flows, which will significantly improve the quality and accessibility of health care. However, because of the ongoing transition process, financial activity in health

care facilities remains the same, that causes inappropriate and irrational resource utilization and is subject to careful analysis and further advanced academic research.

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REVIEW ARTICLE
PRACA POGLĄDOWA

THE PROCESS OF SCIENTIFIC KNOWLEDGE INTEGRATION IN CRIME PREVENTION AND TRENDS OF MEDICAL CRIMINALISTICS DEVELOPMENT IN UKRAINE IN XIX – EARLY XX CENTURY

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ABSTRACT

The aim: To carry out a historical review of the formation and development of medical forensics in the XIX – early XX century in Ukraine, as well as to identify its trends at the present stage.

Materials and methods: The article is based on the study of archival and published materials, the results of integrating expert judgments, judicial practice and regulatory acts of the Ministry of Health of Ukraine, interviewing investigators and court experts. In the course of the research a system of scientific methods has been used: historical, comparative, terminological analysis, formal-logical, sociological.

Conclusions: In the XIX and early XX centuries, the foundations for the formation of medical criminalistics were laid, which facilitated the development of forensic medicine, criminalistics and forensic examination. Special medical knowledge is widely used in investigative, judicial and expert practice. The synthesis of medical and forensic knowledge enhanced the development of a method of personal identification, based on his/her genetic properties, the formation of molecular genetic examination, the study of individual objects at the modern level.

KEY WORDS: Medical Criminalistics, criminalistic knowledge, medical knowledge, Forensic Sciences, Forensic Medicine, molecular genetic expertise

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INTRODUCTION

An important trend of Criminalistics, as a system of scientific knowledge, is its integration, which helps to find optimal solution to the problems of crime prevention. Integration processes in criminalistics are connected with the convergence of sciences, along with opposing trends of differentiation [1] Criminalistics is a special science, which emerged due to the implementation of the achievements of natural, engineering and other sciences (physics, chemistry, biology, toxicology, medicine, pharmacology, psychology, mathematics, etc.) in the practice of combating crime [2].

The development of criminalistics in different countries of the world can be traced in its directions or in the formation of independent academic disciplines – Forensic Toxicology, Forensic Chemistry, Forensic Medicine, Forensic (Criminal) Psychology, Forensic Pharmacology and Forensic Sciences.

The history of borrowing medical knowledge (knowledge of doctors) for forensic purposes is quite interesting. E. Knobloch aptly notes that close cooperation of doctors and criminalists, mainly in the course of collecting material evidence and its evaluation, led to the fact that doctors had to apply their professional knowledge in accordance with the objectives of criminal tactics, and therefore had to improve knowledge in this direction and develop it in detail.

Thus, according to the scholar, a new, special branch of forensic science that can be called Medical Criminalistics, emerged. The work of a forensic doctor in this direction became extremely important, since the times, when forensic medicine limited its activity only to the answers that doctors provided in courts, as experts, were over [3].

THE AIM

To carry out a historical review of the formation and development of medical criminalistics in the XIX – early XX century in Ukraine, as well as to identify its trends at the present stage.

MATERIALS AND METHODS

The article is based on the study of archival and published materials, the results of expert judgment analysis (75 conclusions of forensic medical examinations were studied), judicial practice (electronic registry analysis) and normative legal acts of Ukraine (the normative regulations, which regulate forensic medical research, involvement of specialists-doctors and forensic experts), interviewing investigators (120 investigators of pre-trial investigation institutions) and court experts (82 persons).

In the course of the research a system of scientific methods was used: historical, comparative, terminological analysis, formal-logical, sociological.

REVIEW AND DISCUSSION

Medical Criminalistics reflects a specific link between the knowledge of forensic medicine and the theory of judicial evidence. S. Potapov noted that the subject of forensic research includes any objective and real facts of living reality, which, according to the theory of evidence, may be used as judicial evidence [4].

In contemporary literary sources there were discussions about the attempt to single out "evidence-based forensic medicine". According to scientists, "Evidence-based forensic medicine can be considered as a subdiscipline of forensic medicine. It develops and applies specific methods of expert observation (forensic technologies), which enable experts to draw real conclusions, minimizing the number of systematic and accidental mistakes" [5].

Medical Criminalistics is a complex field of knowledge used in the course of legal activity – the disclosure and investigation of crimes or criminal proceedings. The use of the Medical Criminalistics data is related to the study of specific material evidence in criminal proceedings, the effects of mechanical, electrical, ballistic, or other influence on the person and his/her body; solution of complex medical criminalistic problems in the course of investigating serious and particularly serious violent and other crimes (murder, rape, serious bodily injury, terrorist acts, etc.), the need for professional assistance to investigators, prosecutors or court specialists, doctors, forensic experts and in the course of conducting specific investigatory (search) or judicial actions. Medical Criminalistics is a system of scientific knowledge based on the identification (or diagnostic or situational) research of objects – living persons, corpses (parts of them), material evidence.

The terms "Forensic Medical Criminalistics" and "Medical Criminalistics" are used in the current legal act – Decree of the Ministry of Health Care of Ukraine # 6 of January 17, 1995 (registered by the Ministry of Justice of Ukraine on July 26, 1995 under No. 248 / 784) "On the Development and Improvement of the Forensic Medical Service of Ukraine" and Regulations for the Presentation of Forensic Medical Examination (Researches) in the Departments of Forensic Medical Criminalistics of the Bureau of Forensic Medical Examination. In particular, § 1.2. of the Regulation states that examinations in the department are conducted with the purpose of determining the tools of the trauma, their differentiation and identification on the basis of studying body injuries, clothing, and footwear of the victim. In addition, the examinations are carried out in order to identify the person, determine the nature and elemental composition of micro-objects, traces, overlays, reconstruction of the situation, in which the injury was inflicted. § 1.3. of the Regulation emphasizes that knowledge in the field of medical forensics is used to achieve the goal and special laboratory research methods (anthropological, biophysical, technical, photographic, X-ray, spectral, mathematical, computer, etc.) are applied. In this regard Trace Evidence Examination (researches of traumas, weapons, guns and implements of injuries) (clause 2.2.1.); Examination of Firearms Injuries (Ballistic) (clause 2.2.2.); Person Identification Examination (Osteological and Anthropological) (clause 2.2.3); Micrological Examination (Micro-deposit Research and Elemental Composition of Objects of Forensic Medical Examination)) (paragraph 2.2.4.) [6] are distinguished.

The use of special medical knowledge in criminal proceedings has a long history. H. Gross emphasized that knowledgeable professionals, whose conclusions often have a decisive influence on the case, provide the most important aid, available to a forensic investigator [7].

The definition of experts was provided in the Statute of Criminal Proceeding (1864). Art. 325 of the statute states that knowledgeable professionals are involved in cases, when special expertise or experience in science, art, crafts, industry or an occupation are needed for understanding the case circumstances,. Article 326 of the Statute states that doctors, pharmacists, professors, teachers, technicians, artists, artisans, treasurers and persons who have received special experience as part of their occupation or service can be involved for these purposes [8]. The trust to knowledgeable individuals as reliable sources of special information in the public consciousness is formed as they gain the reputation of people who honestly and professionally fulfill their duty. Distinterestedness of the knowledgeable professionals in the case and their competence became, of course, the most important qualities that the professionals should have... [9]

The formation of medical criminalistics is related to the practice of involving specialists in the field of medicine, medical officers in the investigation of crimes, conducting expert examinations (crime scene examinations, surveys, necroscopy), as well as conducting special blood, saliva, sperm tests, hair analysis, etc. Scientific synthesis and thesis defense are important in this sense. In particular, this concerns the presentation of the Master thesis by a well-known lawyer, specialist in the field of criminal proceedings L. Vladimirov "On the Value of Medical Doctors as Experts in Criminal Proceeding" (1869) [10]. In that period, doctoral dissertations were defended by representatives of the Imperial Kharkov University, specialists in the field of medicine: F. Han as an anatomist of the Anatomy Department "On Forensic Medical Examination of Blood-stain Patterns" (1866); M. Obolonskiy as an anatomist of the Forensic Medicine Department "On Hairs in Forensic Medical Value" (1886); S. Dvornichenko as privatdozent of Forensic Medicine Department "On the Question of Differences in the Blood of Human and Animal in Forensic Medical Value" (1893); N. S. Bokarius as an anatomist of the Forensic Medicine Department "Crystals of Florence's Chemical Nature and Forensic Medical Value" (1902).

Well-known scientific works of N. S. Bokarius: "On Importance of Ligature Strangulation during Hanging" (1904); Forensic Medical Microscopic and Microchemical Examination of Material Evidence (1910); Forensic Medicine in Noted to Lawyers (1915); Priority Simple Examination of the Dead Body during the Militiaman and Intelligence Inquiry (1925); Simple Examination of the Dead in the Crime Scene or its Recognition (1929) etc. are

examples of the development of both Forensic Medicine and Medical Criminalistics.

N.S. Bokarius' scientific interest in forensic medicine regarding the study of material evidence was also reflected in the works of his colleagues, which were carried out under his direct supervision in the early XX century. (18 works, which belong to this section). This applies to the study of blood spots (N. Asvadurova, NN Bokarius, A. Borakovskiy, S. Kaplan, V. Krainskaya-Ignatova, V. Rabinovich, R. Rosenberg), sperm spots (A. Borakovskiy, etc.), hair (N.N. Bokarius, A. Domantovich, N. Ivanitskiy, Y. Tokarev) [11].

The development of medical criminalistics promoted the formation of the theory and practice of forensic examination, creation of special scientific institutions for the implementation of expert functions, the development of scientific and technical methods and approaches to investigating crimes, "for conducting various kinds of scientific and technical research in court cases" [11]. In particular, Offices of Scientific and Forensic Examination in Ukraine were established in Kharkiv, Kyiv and Odesa (Decree of the Council of People's Commissars of the Ukrainian Social Soviet Republic dated July 10, 1923). At the same time, the initiative to create such offices was launched by a famous forensic medicine expert, Professor N. S. Bokarius. It is noteworthy that at that time offices provided for four sections: 1) chemical and physical-chemical research; 2) forensic photographic research; 3) forensic macro- and microscopic research; 4) person identification [12].

The development of the institution of special medical and forensic knowledge, their role in investigating crimes has been confirmed by investigative practice. The results of questioning the investigators of the bodies of pre-trial investigation of the Ministry of Internal Affairs of Ukraine show that in the process of investigating such categories of crimes as serial sexual sadistic murders it is expedient to appoint a forensic examination: forensic medical (indicated by 90, 5% of respondents); forensic psychiatric (indicated by 74, 3% of respondents); forensic biological (indicated by 66, 7% of respondents); forensic psychological (indicated by 55, 2% of respondents); fingerprint exam (indicated by 46, 7% of respondents); forensic sexological (indicated by 40% of respondents); trace examination (indicated by 34, 3% of respondents); forensic portrait examination (indicated by 29, 5% of respondents); forensic pedological (indicated by 26.7% of respondents); psychophysiological (indicated by 25, 7% of respondents); DNA fingerprinting (indicated by 23.8% of respondents); forensic ballistic (indicated by 12.4% of respondents); forensic botanical (indicated by 7, 6% of respondents); etc (4, 8%) [13]. At the same time, 82% of respondents consider it necessary to conduct complex medical and criminalistic examinations.

The foundations of medical criminalistics are reflected in modern forensic research studies – identification of a person on the basis of his/her genetic properties, molecular genetic examination. Today there are new technologies for determining the molecular genetic structure of samples using different methods [14]. I. Perepechina emphasizes that one of the most important achievements in the field of forensic identification over the last three decades is the development and introduction of DNA research methods into practice of the disclosure and investigation of crimes [15]. I. Soltyszewski writes that over the past 25 years, the results of DNA research have taken central stage among important pieces of evidence ... At present, the possibility of using the method of DNA profiling to investigate crimes is widely discussed, that is, to obtain information on the offender's phenotype, his/her race, eye and skin colour etc. The efficiency of this process implies the creation of national DNA databases, international cooperation in the field of DNA data exchange. In practical terms, European standards for these studies are being established and requirements for expert laboratories are being set out. [16]. Genotyposcopic research is relevant in the context of Russia's armed aggression against Ukraine, which necessitates identification of dead soldiers and civilian casualties.

The study of individual biological objects at the micro-or ultraspectral levels (for example, human hair analysis) should also be taken into consideration for identification purposes. This refers to modern scientific achievements in the USA [17]. At the present stage, the formation of the so-called forensic (criminalistic) archeology – a field of knowledge based on methods of forensic science, medicine and archeology – is being developed to help obtain data and extract evidentiary information from excavations after significant time intervals (Great Britain, Poland, USA). Researchers have already started discussing the problems of forensic archeology at international scientific events [18].

CONCLUSIONS

In the XIX and early XX centuries, the foundations for the formation of medical criminalistics were laid, which facilitated the development of forensic medicine, criminalistics (forensic science) and forensic examination. In fact, at that time the scientific school of Prof. N. S. Bokarius was created. Special medical knowledge is widely used in investigative, judicial and expert practice. The formation of medical criminalistics is connected with the necessity of involving experts in the field of medicine to investigate crimes, carrying out special blood, saliva, sperm, hair tests etc. The synthesis of medical and forensic knowledge enhanced the development of a human identification method on the basis of his/her genetic properties, the development of molecular genetic examination, the study of individual objects at the modern level. The use of medical and forensic knowledge has also led to the formation of Forensic (Criminalistic) Archeology.

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REVIEW ARTICLE
PRACA POGLĄDOWA

STEROIDY PŁCIOWE I ADIPOKINY U MĘŻCZYZN Z RAKIEM STERCZA I ICH POWIĄZANIA Z OTYŁOŚCIĄ I ZESPOŁEM METABOLICZNYM

SEX STEROIDS AND ADIPOKINES IN MEN WITH PROSTATE CANCER AND THEIR RELATIONSHIP WITH OBESITY AND METABOLIC SYNDROME

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STRESZCZENIE

Rak gruczołu krokowego jest najczęściej rozpoznawanym nowotworem u mężczyzn na świecie, a w Polsce wśród mężczyzn chorujących na nowotwór stanowi drugą co do częstości przyczynę zgonów. Etiologicznie otyłość wiąże się z rakiem stercza. Podwyższone BMI koreluje z agresywną postacią choroby oraz z większym ryzykiem nawrotu i śmiertelności. Uważa się, że przyczyną są zaburzenia gospodarki hormonalnej, zwłaszcza w zakresie steroidów płciowych, przewlekły stan zapalny z nieprawidłowym wytwarzaniem adipokin, oporność na insulinę z hiperinsulinemią oraz stres oksydacyjny. Wydaje się, że rozpoznanie zespołu metabolicznego może być przydatne w globalnej ocenie rokowania u pacjentów z rakiem stercza.
Celem pracy jest przedstawienie aktualnego stanu wiedzy na temat powiązań pomiędzy otyłością i zespołem metabolicznym a steroidami płciowymi i adipokinami u mężczyzn z rakiem stercza.

SŁOWA KLUCZOWE: rak stercza, steroidy płciowe, adipokiny, otyłość, zespół metaboliczny

ABSTRACT

Prostate cancer is the most commonly diagnosed cancer among men in the world and in Poland it is the second cause of death in men suffering from cancer. Recent evidence suggests that obesity is associated with prostate cancer. Increased BMI correlates with aggressive disease and with higher risk of recurrence and mortality in prostate cancer patients. Obesity can promote the progression of prostate cancer through endocrine disturbances, mainly in sex steroids, through chronic inflammation resulting in altered production of adipokines, peripheral insulin resistance with hyperinsulinemia and oxidative stress. Diagnosis of metabolic syndrome can be used in the global assessment of prognosis in patients with prostate cancer.
The aim of the paper is to present current state of knowledge about connections between obesity, metabolic syndrome, sex steroids and adipokines in men with prostate cancer.

KEY WORDS: prostate cancer, sex steroids, adipokines, obesity, metabolic syndrome

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WSTĘP

ETIOLOGIA RAKA STERCZA

Rak stercza jest obecnie uznany za jeden z największych problemów medycznych na świecie. W Europie jest to najczęstszy nowotwór lity, rozpoznawany w 214 przypadkach na 1000 mężczyzn, przewyższając liczbę nowotworów płuc i jelita grubego [1]. Rak gruczołu krokowego jest drugą co do częstości przyczyną zgonów wśród chorób nowotworowych u mężczyzn [2]. W Polsce w 1985 roku stwierdzono 2010 nowych zachorowań, a w roku 2013 już 12162. W ciągu niespełna trzydziestoletniego okresu obserwacji wykrywalność raka stercza zwiększyła się w naszym kraju o ponad 600% i obecnie jest on drugim najczęściej występującym nowotworem u mężczyzn (15,5%) [3].

Etiologia raka prostaty jest niejednorodna. Do niemodyfikowalnych czynników ryzyka należą: wiek, wywiad rodzinny oraz rasa. 87% zachorowań w Polsce występuje po 60. roku życia, a maksimum zachorowań obserwuje się po 75. roku życia (około 350 / 100 000) [4]. Analizując etiopatogenezą raka stercza, coraz większą uwagę zwraca się na modyfikowalne czynniki stylu życia, takie jak aktywność fizyczna i dieta. Wyższa częstość występowania raka stercza jest związana z modelem "zachodniego" stylu życia [5].

CEL

Celem pracy jest przedstawienie aktualnego stanu wiedzy na temat powiązań pomiędzy otyłością i zespołem metabolicznym a steroidami płciowymi i adipokinami u mężczyzn z rakiem stercza.

PRZEGŁĄD I DYSKUSJA

ANDROGENY

Endogenny testosteron jest głównym regulatorem wzrostu i funkcjonowania stercza. Między 10. a 20. rokiem życia, podczas okresu znacznego wzrostu stężenia testosteronu, dochodzi do rozwoju prostaty. Za stężenie testosteronu we krwi odpowiedzialna jest przede wszystkim oś podwzgórze-przysadka-jądra/nadnercza, podlegająca mechanizmowi ujemnego sprzężenia zwrotnego. Stężenie testosteronu zależy także od masy tkanki tłuszczowej i jej funkcji. W komórkach prostaty testosteron redukowany jest pod wpływem 5-alfa-reduktazy do dihydrotestosteronu. Receptory androgenowe wiążą zarówno testosteron, jak i dihydrotestosteron, przy czym ten ostatni ma większe powinowactwo do receptorów [6]. Ciągle badana jest rola testosteronu i dihydrotestosteronu (DHT) jako induktorów nowotworu stercza. Według Nishiyama i wsp. stężenie DHT w komórkach stercza nie koreluje z poziomem testosteronu w osoczu. U mężczyzn z rakiem prostaty po ablacji androgenowej wewnątrzsterczowy poziom DHT pozostaje na poziomie 25% stężenia wyjściowego. Dowodzi to roli konwersji hormonów nadnerczy w utrzymaniu lokalnego stężenia DHT [7, 8].

Androgeny uważa się za decydujący czynnik w rozwoju i wzroście komórek stercza, ale także w rozwoju raka gruczołu krokowego [9, 10]. Zależności pomiędzy rakiem stercza i stężeniami androgenów zostały opisane przez Hugginsa i Hodgesa już w latach 40. [11]. W pracy, za którą otrzymali w 1966 roku Nagrodę Nobla wykazali, że kastracja powoduje regresję raka stercza, natomiast testosteron jego progresję. Wnioski o androgenozależnym charakterze raka stercza spowodowały, że terapia antyandrogenowa stała się złotym standardem leczenia zaawansowanego i przerzutowego raka prostaty [12, 13]. Niestety, mimo uzyskania kastracyjnego poziomu testosteronu, rak stercza ulega progresji i przejściu w stadium opornego na kastrację nowotworu, po średnim okresie od 12 do 33 miesięcy [14]. Średnia przeżycia chorych z przerzutowym rakiem stercza, opornym na kastrację, została określona na nie więcej niż 25 miesięcy [15].

Pomimo teorii Hugginsa i Hodgesa na temat wpływu testosteronu na rozwój raka prostaty, większość prac oceniających prospektywnie i retrospektywnie powiązania raka prostaty ze stężeniami endogennego testosteronu, DHT lub wolnego testosteronu, nie wykazała statystycznie istotnych zależności [16]. Wyniki badań populacyjnych wskazują na to, że wysokie stężenia endogennego testosteronu nie zwiększają ryzyka raka gruczołu krokowego, a niskie stężenia testosteronu paradoksalnie kojarzą się z gorszym rokowaniem, z wyższym wskaźnikiem agresywności raka ocenianej w biopsji stercza za pomocą skali Gleasona oraz z większym ryzykiem nawrotu po przeprowadzonym leczeniu operacyjnym [17]. W ostatnim czasie teoria dotycząca kluczowej roli testosteronu w patogenezie raka stercza została zastąpiona przez koncepcję złożonych, hormonalnych oddziaływań, w których priorytetową rolę odgrywa estradiol, metabolit testosteronu, oddziaływujący synergistycznie z leptyną i insuliną oraz SHBG. Wykazano, że pomiędzy sygnalizacją receptorów androgenowych i metabolizmem lipidów istnieją silne powiązania. Szybko rosnące komórki nowotworu wykazują zwiększone zapotrzebowanie na energię, którą uzyskują poprzez zwiększoną syntezę kwasów tłuszczowych i cholesterolu [18]. Badanie Gann i wsp. [19] sugeruje, że wysoki poziom testosteronu i niski poziom SHBG są związane ze zwiększonym ryzykiem raka prostaty.

ESTROGENY

Zależności między testosteronem, estradiolem i częstością występowania raka stercza są przedmiotem wielu analiz. Zarówno w prawidłowej jak i w nowotworowej tkance stercza stwierdza się ekspresję receptorów estrogenowych. Sygnalizacja ERα sprzyja kancerogenezie, podczas gdy sygnalizacja ERβ jest antyproliferacyjna, proapoptotyczna i ochronna [20, 21]. Estradiol powstaje w wyniku konwersji testosteronu, przy udziale kompleksu enzymatycznego aromatazy, związanego z cytochromem P450. Aromataza P450 jest obecna przede wszystkim w adipocytach, komórkach gruczołu krokowego i komórkach endotelialnych. Czynnikami stymulującymi aktywność aromatazy są: wytwarzany w tkance tłuszczowej kortyzol, insulina, leptyna oraz wysokie poziomy glukozy [22]. Zwiększenie aktywności aromatazy pobudza produkcję estrogenów, które poprzez sygnalizację związaną z receptorami ERα, nasilają proliferację i wzrost komórek we wrażliwych tkankach. W efekcie przemian steroidów płciowych lokalne poziomy estrogenów w tkankach obwodowych mogą być wysokie. Należy zaznaczyć, że stężenie steroidów płciowych we krwi nie odzwierciedla w sposób prosty ich aktywności tkankowej. Wprawdzie wykazano istnienie powiązań pomiędzy polimorfizmami w obrębie genu CYP19 a stężeniem testosteronu, estrogenu i SHBG we krwi [23], to jednak miejscowe oddziaływanie steroidów zależy też w dużej mierze od gęstości i wrażliwości receptorów.

Udowodniono, że stan zapalny obecny w otyłości nasila aktywność aromatazy [24] oraz pobudza ekspresję CYP1B1 – enzymu, który indukuje niekorzystną hydroksylację estrogenów do karcynogennych pochodnych. Proces ten określany jest mianem adipogenotoksykozy [25].

CECHY KOMÓREK NOWOTWOROWYCH RAKA STERCZA

Komórki nowotworowe raka stercza charakteryzują się niekontrolowaną proliferacją, inwazyjnością i zdolnością do przerzutowania. Cechy te są wtórne do zmian w czynnikach wzrostu i ich receptorach oraz wewnątrzkomórkowych szlakach sygnalizacyjnych [26]. Komórki raka wykazują również odmienny metabolizm i mają inne zapotrzebowanie energetyczne w porównaniu do komórek zdrowych. Wyniki badań metabolicznych wykazały,

iż komórki raka prostaty charakteryzują się pobudzoną lipogenezą, cholesterologenezą i cyklem Krebsa. Najważniejszym z tych procesów wydaje się zwiększona lipogeneza, która przeprogramowuje metabolizm raka stercza. Kluczowym enzymem, którego wysoka ekspresja wyróżnia komórki raka prostaty, jest syntaza kwasów tłuszczowych FAS (*fatty acid synthase*). W kancerogenezie jest też aktywowany SREBP-1c, białko wiążące sekwencje odpowiedzi na sterole-1c (*sterol-regulatory-element-binding protein*--1c), który jest silnym stymulatorem lipogenezy. Synteza *de novo* kwasów tłuszczowych umożliwia komórkom nowotworowym proliferację, inwazję i przerzutowanie [27]. Markerami lipogenezy są cholina i octan, których analogi, czyli 11C-octan i 11C-cholina są wykorzystywane w technice PET do obrazowania zwiększonej aktywności proliferacyjnej, a tym samym w ocenie zaawansowania, prognozowania i monitorowania odpowiedzi na leczenie.

Nie do końca wiadomo, czy zaburzenia metaboliczne obecne w komórkach raka stercza są identyczne z uogólnionymi zaburzeniami w tkance tłuszczowej. Uważa się, że istotną rolę w etiopatogenezie raka stercza odgrywają różne zmiany w mikrośrodowisku, które wynikają z interakcji między komórkami nowotworowymi i zrębowymi [28]. W ostatnim czasie badania naukowe koncentrują się wokół cytokin, chemokin i adipokin, których stężenia w surowicy zmieniają się w otyłości i które korelują z obecnością i progresją raka prostaty.

STYL ŻYCIA, OTYŁOŚĆ I ZESPÓŁ METABOLICZNY JAKO CZYNNIKI ETIOLOGICZNE RAKA STERCZA

Rozwojowi raka prostaty oprócz nadmiernego gromadzenia tkanki tłuszczowej sprzyja nieprawidłowy styl życia. Prawdopodobieństwo rozwoju raka jest zwiększone u mężczyzn prowadzących niską aktywność fizyczną, palaczy tytoniu, spożywających duże ilości zwierzęcych tłuszczy nasyconych, czerwonego mięsa, jajek i choliny. U tych samych mężczyzn stwierdzono również większe ryzyko postaci raka stercza o gorszym rokowaniu. Z kolei czynnikami o właściwościach protekcyjnych, oprócz zwiększonej aktywności fizycznej, są spożywanie dużych ilości ryb i warzyw, zwłaszcza pomidorów [29]. Badania sugerują, że ilość tłuszczu w diecie wpływa nie tylko na rozwój, ale też na stopień agresywności raka stercza [30].

Dane z piśmiennictwa sugerują, że dieta bogata w kwasy omega-3 istotnie zmniejsza objętość gruczołu krokowego i redukuje ekspresję makrofagów M1 i M2 oraz cytokin i chemokin CCL-2 [31], natomiast dieta o wysokim indeksie glikemicznym zwiększa ryzyko zachorowania na raka prostaty u mężczyzn [32].

Ogniwa łączące rodzaj diety i kancerogenezę pozostają w dużej mierze nieznane. Proponowane mechanizmy leżące u podstaw raka stercza indukowanego przez dietę wysokotłuszczową można podzielić na związane z sygnalizacją czynników wzrostu, metabolizmem lipidów, efektami prozapalnymi i modulacją hormonalną.

Chociaż dieta jest niezależnym czynnikiem ryzyka raka prostaty, to te same mechanizmy mogą wyjaśniać patofizjologię nowotworzenia u pacjentów z nadwagą i otyłością [33]. Wykazano, że nadmiar tkanki tłuszczowej, która jest złożonym, aktywnym, hormonalnie i metabolicznie organem, etiologicznie wiąże się z około 20% wszystkich nowotworów [34]. Według danych Krajowego Rejestru Nowotworów nadwaga i otyłość są przyczyną 11% zachorowań na raka okrężnicy, 9% przypadków raka piersi, 39% raka trzonu macicy, 37% gruczolakoraka przełyku, 25% nowotworów nerki i 24% nowotworów pęcherzyka żółciowego [4]. Dane epidemiologiczne wskazują, że wzrost BMI tylko nieznacznie zwiększa ryzyko rozwoju raka prostaty, ale zależności te są wyraźniejsze w przypadku postaci zaawansowanych. Wykazano, że otyłość trzewna wiąże się z większym stopniem agresywności raka gruczołu krokowego i u osób otyłych częściej występują przerzuty oraz większa jest umieralność z powodu tego nowotworu [35, 36].

U osób otyłych częściej niż w ogólnej populacji występuje zespół metaboliczny. Wynika on z narastającej masy ciała, dysfunkcji tkanki tłuszczowej i oporności insulinowej. W badaniu Me-Can (*Metabolic Syndrome and Cancer Project*) przeprowadzonym na ponad 6,6 tys. mężczyzn z rakiem prostaty skojarzenie podwyższonego ciśnienia tętniczego oraz zaburzonych parametrów metabolicznych korelowało ze zwiększoną śmiertelnością z powodu tego nowotworu [37]. Wykazano, że osobnicy z zespołem metabolicznym nie tylko są narażeni na rozwój raka prostaty, ale też jelita grubego, piersi i endometrium [38]. Gomez i wsp. dowodzą, że zespół metaboliczny nie tylko zwiększa ryzyko występowania raka stercza, ale wiąże się z jego większą agresywnością [39].

Zasadniczym ogniwem łączącym otyłość, zespół metaboliczny i kancerogenezę są metaboliczne i endokrynologiczne następstwa nadmiernej akumulacji tkanki tłuszczowej. Steroidy płciowe oddziaływujące na tkankę tłuszczową i metabolizowane w jej obrębie stanowią najpewniej czynniki zaangażowane w złośliwą transformację.

Insulinooporność występująca w zespole metabolicznym jest stanem zmniejszonej reaktywności tkanek na fizjologiczne poziomy insuliny. Na poziomie molekularnym oporność jest wywołana dysregulacją kaskady pobudzeń indukowanych przez insulunę. W oporności insulinowej zwiększa się produkcja IGF-1 i IGF-2 (insulinopodobnych czynników wzrostu typu 1 i 2), które aktywują receptor IRS-1, stymulują szlak sygnałowy MAPK (kinaza białkowa zależna od mitogenu), PKB i mTOR. W konsekwencji zwiększa się aktywność anaboliczna, antyapoptotyczna i mitotyczna komórek nowotworowych i stymulowane są nasilone podziały komórkowe. IGF-1 pobudza też aktywność genu Ras, który odgrywa dużą rolę na początkowych etapach kancerogenezy [40].

Wykazano, że otyłość, przewlekła hiperinsulinemia i insulinooporność sprzyjają rozwojowi raka prostaty. Duża prospektywna analiza wykazała, że wyższe stężenia peptydu C w surowicy były ściśle związane ze zwiększoną śmiertelnością u chorych z rakiem stercza [41], a wysoki poziom insuliny oraz aktywacja osi insulina i IGF-1/IGF-1R poprzez sygnalizację kinazy tyrozynowej zwiększały progresję

raka. Pobudzenie receptorów kinazy tyrozynowej skutkuje aktywacją kaskady białek wewnątrzkomórkowych i zmianą ekspresji genów syntezy białek [30].

Oporności insulinowej towarzyszy zmniejszona wątrobowa produkcja białka wiążącego hormony płciowe (SHBG) i niskie stężenie testosteronu. U mężczyzn androgeny wpływają na masę tkanki tłuszczowej trzewnej, a więc niski poziom testosteronu może w konsekwencji doprowadzić do przyrostu masy i zmiany rozmieszczenia tkanki tłuszczowej. W hipogonadyzmie męskim często występuje otyłość brzuszna, a suplementacja testosteronem zmniejsza masę tłuszczu [42, 43].

U osobników męskich testosteron i DHT hamują różnicowanie preadipocytów, a efekt jest najsilniejszy w tkance tłuszczowej trzewnej. Poza tym steroidy nasilają lipolizę zależną od katecholamin, regulują aktywność β-AR i α2-AR,wpływają na aktywność PKC oraz drogi przekaźnictwa MAPK i c-fos [44].

Androgeny swoje działanie wywierają poprzez jądrowe receptory androgenowe, aczkolwiek efekty niegenomowe również mają znaczenie. Testosteron podczas kontaktu z komórką tłuszczową zwiększa liczbę receptorów androgenowych. Receptory mogą też wiązać estradiol i progesteron, chociaż w dużo mniejszym stopniu. Receptory androgenowe są obecne w adipocytach, preadipocytach i w podścielisku. Testosteron poprzez receptory hamuje aktywność LPL i wychwyt WKT, a w rejonach trzewnych efekt ten jest najwyraźniejszy. Jednocześnie steroid pobudza aktywność HSL w adipocytach trzewnych i w efekcie lipolizę, zarówno podstawową, jak i stymulowaną katecholaminami [45].

U otyłych mężczyzn z opornością insulinową i hiperinsulinemią oraz z niskim stężeniem testosteronu, ryzyko raka stercza jest wyższe niż u mężczyzn z prawidłowym BMI. Boehm i wsp. [46] analizowali zależności między otyłością i słabo zróżnicowanym rakiem gruczołu krokowego. Autorzy wykazali, że pacjenci z podwyższonym obwodem w talii mieli częściej rozpoznawanego nisko zróżnicowanego raka stercza.

ADIPOKINY

Otyłość lub zespół metaboliczny mogą sprzyjać rozwojowi raka prostaty poprzez działanie adipokin [47].

ADIPONEKTYNA

Adiponektyna jest 30-kDa białkiem zbudowanym z dwóch domen o różnej strukturze: włóknistej podobnej do kolagenu i globularnej, homologicznej strukturalnie z kolagenem X, VIII oraz z czynnikiem dopełniacza C1q. Białko jest produkowane przez podobne, różnicujące się adipocyty, a ekspresja genu adiponektyny wzrasta podczas dojrzewania preadipocytów nawet 100-krotnie. Ekspresję genu adiponektyny pobudzają czynniki transkrypcyjne: SREBP-1c, C/EBP, PPAR.

Sekrecja nasila się również pod wpływem insuliny i IGF-1, które pobudzają SREBP-1c. Istotną rolę odgrywa rodzaj diety: dieta wysokotłuszczowa hamuje sekrecję adiponektyny. Produkcję adiponektyny hamują glikokortykoidy, katecholaminy oraz cytokiny zapalne [48].

Korzystne efekty adiponektyny można podzielić na: metaboliczne, ogólne, naczyniowe i przeciwnowotworowe. Adiponektyna w mięśniach szkieletowych nasila ekspresję GLUT-4 i w konsekwencji wychwyt glukozy, a poprzez AMPK ułatwia przekazywanie sygnałów insuliny. W konsekwencji białko nasila wrażliwość na insulinę [49]. Adiponektyna zwiększa też ekspresję FAT, wychwyt i oksydację WKT oraz zmniejsza ilość odłożonych w mięśniach lipidów. W wątrobie działa synergistycznie z insuliną, bezpośrednio nasilając jej supresyjne działanie w zakresie produkcji glukozy oraz zwiększając magazynowanie glikogenu. Pod wpływem adiponektyny aktywowana jest AMPK i zmniejsza się aktywność enzymów odpowiedzialnych za glukoneogenezę. Redukcji ulega wychwyt kwasów tłuszczowych i gromadzenie TG. Adiponektyna hamuje w wątrobie glukoneogenezę, lipogenezę oraz zwiększa utlenianie kwasów tłuszczowych. W komórkach tłuszczowych adiponektyna nasila transport glukozy oraz hamuje lipogenezę podstawową i stymulowaną insulina [50]. Oprócz właściwości przeciwcukrzycowych adiponektyna wykazuje efekty przeciwzapalne. Za pośrednictwem IL-2 adiponektyna hamuje aktywację komórek NK i limfocytów T oraz proliferację mielomonocytowych komórek progenitorowych. Białko indukuje wydzielanie cytokin przeciwzapalnych IL-10 i IL-1RA w monocytach, makrofagach i w komórkach dendrytycznych [51]. Adiponektyna wiąże się specyficznie z komórkami endotelium, a w przypadku jego uszkodzenia gromadzi się w przestrzeni subendotelialnej. Zmniejszenie stężenia adiponektyny jest silnie związane nie tylko z opornością na insulinę, otyłością i cukrzycą typu 2, ale także z większym ryzykiem różnych typów nowotworów [52].

W większości piśmiennictwa wykazano odwrotną korelację między adiponektyną a ryzykiem nowotworu gruczołu krokowego. Wiele prac dowodzi, że poziomy adiponektyny są niższe u pacjentów z rakiem stercza i odwrotnie proporcjonalne do stopnia zaawansowania tej choroby [52–54].

Badania *in vitro* wykazały, że adiponektyna hamuje wzrost i proliferację komórek raka stercza oraz antagonizuje proliferacyjne działanie leptyny i IGF-1 w niezależnym od androgenów raku gruczołu krokowego [55]. Tan i wsp. wykazali, że istotne obniżenie stężenia adiponektyny prowadzi do zwiększonej proliferacji komórek guza i inwazji [56]. Duże prospektywne badanie dotyczące poziomów adiponektyny w osoczu i ryzyka raka gruczołu krokowego wykazało, że mężczyźni z wyższymi stężeniami krążącej adiponektyny mieli zmniejszone ryzyko rozwoju słabo zróżnicowanego raka lub przerzutów [57]. Ponadto wykazano, że leczenie adiponektyną zmniejsza stres oksydacyjny w liniach komórkowych ludzkiego raka stercza, w sposób zależny od dawki [58]. Liczne dowody wskazują, że adiponektyna działa antyproliferacyjnie w komórkach raka prostaty, hamując proliferację komórek aktywowaną dihydrotestosteronem [59]. Wykazano, że nadekspresja adiponektyny w liniach komórkowych raka gruczołu krokowego hamuje proliferację komórek nowotworowych za

pośrednictwem mTOR [60]. Wreszcie, Gao i wsp. wykazali, że mikroRNA jest w stanie stymulować neoangiogenezę w tkankach raka prostaty poprzez obniżenie poziomu receptorów adiponektyny [61]. Kilka polimorfizmów genetycznych jest związanych z predyspozycją do zwiększonego ryzyka raka stercza. W metaanalizie 133 opublikowanych badań, warianty AdipoQ rs2241766 i AdipoR1 rs10920531 były związane z wyższym ryzykiem nowotworu gruczołu krokowego. Odwrotnie, wariant AdipoR1 rs2232853 wiązał się z mniejszym ryzykiem rozwoju tego typu nowotworów złośliwych [62]. Trzy powszechne polimorfizmy AdipoQ oceniono w dużej kohorcie pacjentów z miejscowym rakiem stercza, którzy przeszli radykalną prostatektomię. Allel rs182052 AdipoQ był związany zarówno z wyższym ryzykiem nawrotu biochemicznego, jak i obniżonym poziomem adiponektyny. Analiza wykazała, że korelacja ta była bardziej widoczna u pacjentów z brzuszną otyłością [63].

Niedobór adiponektyny może więc być potencjalnym biomarkerem dla wczesnego wykrywania raka stercza. Zatem podniesienie poziomów adiponektyny u pacjentów z rakiem gruczołu krokowego powinno stać się użytecznym celem terapeutycznym. Niemniej jednak, biorąc pod uwagę fakt, że dane z piśmiennictwa wydają się czasami sprzeczne, konieczne są dalsze badania, zarówno epidemiologiczne, jak i eksperymentalne, w celu wyjaśnienia związku między adiponektyną, a rozwojem nowotworu gruczołu krokowego.

LEPTYNA

Białko leptyny pochodzące z adipocytów ma masę cząsteczkową 16 kDa i jest kodowane na długim ramieniu chromosomu 7 (7q32.1). Leptyna jest wydzielana do krwiobiegu i bierze udział w regulacji procesów energetycznych oraz fizjologicznej masy ciała. Wydzielanie leptyny jest większe w podskórnych komórkach tłuszczowych. Stwierdzono, że stymulacja leptyny działa prozapalnie przez indukcję cytokin takich jak TNFα, IL-1 i IL-6. Stężenie leptyny we krwi zależy od wielu czynników: TNFα, estrogeny, glikokortykoidy i agoniści PPARγ stymulują, natomiast GH i androgeny hamują sekrecję hormonu. mRNA leptyny wzrasta pod wpływem insuliny i maleje wskutek aktywacji receptorów β3-AR. Hiperleptynemia występująca w przypadku oporności receptorów na hormon – charakterystyczna dla osób otyłych może być powiązana z rakiem stercza poprzez proliferację komórkową drogą MAPK [64]. Leptyna stymuluje angiogenezę oraz ma wpływ na rozwój przerzutów nowotworowych. Wykazano, że leptyna działa bezpośrednio na komórki raka prostaty, regulując cykl komórkowy oraz przyczynia się do uwalniania VEGF [65]. Postawiono hipotezę, że obserwowany u otyłych pacjentów niski poziom adiponektyny, przy równoczesnym wysokim poziomie leptyny i rezystyny może zwiększać agresywność raka stercza [61]. Niektóre badania potwierdzają te hipotezy, pokazując, że wysoki poziom leptyny może być skorelowany z wyższym stopniem złośliwości guza i stadium guza, podczas gdy wysokie poziomy adiponektyny były związane z mniej agresywnym stopniem złośliwości guza i stadium choroby [66]. Badania *in vitro* wykazują, że specyficzne polimorfizmy genetyczne leptyny i adiponektyny oraz ich receptorów są związane z progresją raka stercza poprzez nasilenie stanu zapalnego oraz zwiększoną angiogenezę [66].

REZYSTYNA

Gen rezystyny znajduje się na krótkim ramieniu chromosomu 19 (19p13). Ludzka rezystyna jest 10-kDa bogatym w cysteinę polipeptydem syntetyzowanym jako prekursor 108 aminokwasów zawierający sekwencję sygnałową 18 aminokwasów i region dojrzały 90 aminokwasów. Jest ona głównie wydzielana przez adipocyty. Ludzka rezystyna stymuluje wydzielanie prozapalnych cytokin i chemokin, w tym TNFα i IL-2 oraz bierze pośredni udział w patogenezie insulinooporności i cukrzycy typu 2 u otyłych ludzi [67]. Rezystyna jest jednym z czynników wydzielanych przez tkankę tłuszczową powodujących zmianę metylacji DNA i ekspresję genów w tkankach obwodowych, prawdopodobnie wpływając na ekspresję mikroRNA (miR) [68]. Doprowadza to do złośliwej transformacji poprzez zaburzenie równowagi między miR przeciwnowotworowym i onkogennym. Odbywa się to poprzez pobudzenie nowotworowego miR i przez zahamowanie przeciwnowotworowego miR. Rezystyna indukuje niektóre onkogenne miR, które negatywnie wpływają na pewne supresory nowotworów, a zatem działają antyapoptotycznie i sprzyjają przeżyciu. W chorobach nowotworowych wysokie poziomy miR-21 są skorelowane ze słabym przeżyciem i gorszym rokowaniem [69]. Obecnie trwają badania nad stosowaniem różnych inhibitorów miR-21 jako leków przeciwnowotworowych [70]. Wykazano, że długotrwałe hamowanie miR-21 prowadzi do zmniejszenia otyłości [71]. Rezystyna nie tylko indukuje kilka onkogennych miR, ale także hamuje kilka przeciwnowotworowych miR, w tym miR-27b. miR-27b scharakteryzowano jako supresor dla kilku genów związanych z rakiem, w tym PPARγ [72]. Udowodniono, że miR-27b współdziała z lekami przeciwnowotworowymi poprzez aktywację p53. W badaniach *in vitro* wykazano, że rezystyna odgrywa przyzwalającą rolę w proliferacji komórek raka prostaty [73], natomiast wciąż brakuje danych dotyczących stężeń rezystyny u pacjentów z rakiem stercza.

OMENTYNA

Omentyna jest adipokiną o masie 34kDa, wydzielaną głównie przez tkankę tłuszczową trzewną. Pierwszymi komórkami, w których wykazano występowanie omentyny były komórki Panetha, w których omentyna może wiązać się z elementami ściany komórkowej bakterii, stanowiąc w ten sposób element układu odpornościowego błony śluzowej jelita. Kolejne badania wykazały jej udział w zwiększeniu indukowanego przez insulinę wychwytu glukozy przez komórki tkanki tłuszczowej [74]. W tkance osób otyłych oraz chorych na cukrzycę typu 2 obserwuje się obniżenie ekspresji mRNA dla tego białka. Podobny spadek ekspresji wykazano w chorobie Crohna oraz w reumatoidalnym zapaleniu stawów. Przeciwzapalna rola omentyny polega na obniżeniu ekspresji białek CRP, TNF-α i jądrowego czynnika transkrypcyjnego NF-KB [74].

Podwyższone stężenia omentyny w osoczu obserwowano u pacjentów z rakiem okrężnicy [75] oraz u pacjentów z rakiem gruczołu krokowego [76]. Fazeli i wsp.[75] podają, że średnie stężenie omentyny u chorych na raka okrężnicy wynosi 201.37 ng/ml, a u osób zdrowych 8,95 ng/ml. Wyniki te wskazują na znaczny wzrost stężenia tej adipokiny u pacjentów z chorobą nowotworową.

W badaniach *in vitro* wykazano, że podawanie omentyny do hodowli komórek raka wątrobowokomórkowego zwiększa ekspresję białka TP53, bez zwiększenia stężenia jego mRNA, co sugeruje, że wzrost stężenia tego białka może być związany z jego potranslacyjną modyfikacją (acetylacją). Wiadomo, że omentyna hamuje deacetylację białka TP53 poprzez inhibicję działania deacetylazy Sirt1 [77]. Być może wzrost stężenia omentyny opisywany w przypadku raka stercza i okrężnicy stanowi element mechanizmu obronnego organizmu skierowanego przeciwko komórkom nowotworowym. Niektórzy autorzy sugerują, że omentyna może wywierać stymulujący wpływ na progresję choroby nowotworowej poprzez szlak sygnalizacji Akt. Stymulacja proliferacji przez omentynę za pośrednictwem szlaku sygnałowego Akt odbywa się np. w osteoblastach [78].

Niejednoznaczne wyniki badań dotyczące wpływu tej omentyny na przebieg choroby nowotworowej mogą wynikać ze zróżnicowanego podłoża molekularnego poszczególnych nowotworów. Wyniki wielu autorów sugerują, że oznaczenie omentyny w osoczu może mieć znaczenie we wczesnym rozpoznawaniu raka stercza. Dalsze badania mogą w przyszłości dostarczyć nowych informacji na temat diagnozy i terapii raka gruczołu krokowego.

CHEMERYNA

Chemeryna jest adipokiną wydzielaną przez adipocyty 3T3-L1, hepatocyty, komórki jelita cienkiego, nerek oraz trombocyty. Białko jest czynnikiem chemotaktycznym, wspomaga angiogenezę oraz wykazuje właściwości prozapalne. Bierze też udział w adipogenezie, regulacji metabolizmu tkanki tłuszczowej oraz powoduje infiltrację tkanki tłuszczowej przez makrofagi. Rozważana jest też rola chemeryny w kancerogenezie [79]. Wyższe stężenia chemeryny obserwowano u pacjentów z niedrobnokomórkowym rakiem płuca, w porównaniu do grupy kontrolnej [80], podobne różnice stwierdzono wśród chorych na raka żołądka i raka jelita grubego w porównaniu do zdrowej grupy kontrolnej [81]. Wprawdzie nie wykazano różnic w stężeniu chemeryny u mężczyzn z rakiem stercza w porównaniu do łagodnego przerostu prostaty, ale stężenie chemeryny korelowało ze stopniem złośliwości u pacjentów z rakiem [82]. Nieliczne doniesienia na temat chemeryny nie pozwalają jeszcze na jednoznaczne określenie roli adipokiny w patogenezie raka prostaty.

WNIOSKI

Procesy patofizjologiczne występujące w otyłości wpływają na agresywną i przerzutową postać raka stercza. Poznanie tych mechanizmów, a zwłaszcza zaburzeń w zakresie steroidów płciowych u osób otyłych, pozwoliłoby na lepsze zrozumienie przyczyn kancerogenezy i na prognozowanie dalszego przebiegu choroby. W surowicy pacjentów z rakiem prostaty stwierdzono zmienione stężenia różnych adipokin, co sprawia, że mogłyby one stanowić laboratoryjne markery rozwijającego się nowotworu oraz mogłyby być wykorzystane do monitorowania raka stercza. Przy rozpoznaniu raka prostaty ocena profilu metabolicznego może mieć duże znaczenie w określaniu ryzyka rozwoju postaci bardziej agresywnych.

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REVIEW ARTICLE
PRACA POGLĄDOWA

BIOETHICAL PROBLEMS ARISING IN THE STUDY OF SINGLE-NUCLEOTIDE GENE POLYMORPHISMS OF OCCUPATIONAL DISEASES

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ABSTRACT

In Ukraine, about 3 million people work in hazardous and dangerous conditions. The study of hereditary specificity in development of occupational diseases is being actively conducted through molecular genetic analysis of single-nucleotide gene polymorphisms. While studying single-nucleotide gene polymorphisms of occupational diseases, many complicated bioethical questions arise regarding the confidentiality of personal data, the choice between the profession chosen and the risk to one's own health.
Complicated bioethical issues that arise when studying single-nucleotide gene polymorphisms of occupational diseases need to be actively discussed, not only by physicians, occupational pathologists, employers, scientists, but also by politicians and lawyers, taking into account ethical and social norms and implications.

KEY WORDS: single-nucleotide gene polymorphism; occupational diseases; multifactorial diseases; bioethical issues

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INTRODUCTION

In the current state of the industry in Ukraine, the adverse effect of harmful and dangerous production factors on workers in all industries is increasing. The number of productions that do not meet the established standards of the working conditions makes up 35,0-57,7%. The main reasons for such situation are the aging of production facilities, use of outdated technologies and equipment, inadequate use of collective and personal protection [1]. Occupational diseases is a significant medical and social problem because they are one of important indicators of the health of the working population and can be result of the direct impact of harmful production factors and the labor process on the human body [2]. About 3 million people work in unfavorable conditions in Ukraine.

Recently, the attention of scientists is focused on determining the role of the genetic component in the development of multifactorial diseases (MFD), the category of which includes occupational diseases. Occupational diseases is a group of diseases that are complicated by working conditions, and their frequency exceeds that of workers outside the influence of certain occupational harmful factors. Characteristic features of this group of diseases are: high rates of increase in the incidence, disability and mortality of the working age population, as well as significant economic losses in the form of compensation for disability and disability payments. In Ukraine, the list of occupational diseases was approved by the Decree of the Cabinet of Ministers of Ukraine No. 1662 of November 8, 2000, in which the diseases are presented depending the origin of the production factor. In the list of occupational diseases the following nosological types are presented: chronic bronchitis, pneumosclerosis, fibrotic alveolitis, toxic hepatitis, liver damages with development of fibrosis and cirrhosis, osteoporosis, osteosclerosis, diseases associated with the effect of ionizing and others.

MFD is the most common group of human somatic pathology, their frequency is up to 90%. In the pathogenesis of MFD, several genes play a role, realizing pathological effects only under certain adverse environmental conditions. Modern concepts about etiology of most work-related diseases also include provisions on multifactoriality and polygenicity. It is believed that the risk of occupational diseases depends on the individual susceptibility of humans, which is genetically determined by gene polymorphism [3-6]. However, to date, the role of various occupational factors and gene polymorphisms as those that or change the frequency and course of occupationally induced MFD has not been fully elucidated [3, 7].

THE AIM

The purpose of the work was to identify the most relevant bioethical issues that occur in the study of single-nucleotide gene polymorphisms of occupational diseases.

REVIEW AND DISCUSSION

In Ukraine, it was Academician Yuriy Kundiev who began to study the individual susceptibility of persons from

different occupational groups to the risk of developing occupational diseases using molecular genetic methods, and who was the founder and leader of the bioethical movement in Ukraine (Fig. 1).

Currently, molecular genetic markers are the latest tool for epidemiological research in occupational medicine, they are a real basis for identifying occupational groups of individuals with an increased degree of susceptibility to a specific causing disease or harmful production factor. Genetic factors cannot be modified, so researchers have the unique opportunity to study individual differences of the working body associated with a predisposition to certain occupational factors or occupational diseases. At the same time, workers engaged in hazardous and harmful industries are a specific risk group that is exposed to a double stress, from the effects of adverse working conditions and from the environmental pollution [6]. Several approaches are used to estimate the rate of growth of the mutation process and the volume of genetic load in a population of people who have been affected by adverse factors, including professional ones:

- phenotype analysis – studies on the frequency of hereditary diseases, congenital malformations and reproductive function;
- cytogenetic analysis (FISH – fluorescence in situ hybridization) studies on the frequency of unstable and stable chromosomal aberrations, aneuploidy and sister chromatid exchange in human somatic cells;
- molecular genetic analysis – studing gene mutations – gene polymorphisms (SNP – Single nucleotide polymorphism) in somatic cells. SNP is the heterogeneity of the primary DNA structure manifested in single-nucleotide (point) differences of alleles [8].

All scientific studies of the human hereditary material involve obtaining the informed consent from each study participant. The informed consent form is developed for the specific study separately and approved by the Bioethics Commission at the venue. The questionnaire provides an explanation for a patient about the purpose and scope of the study, benefit to the patient, and potential risks involved in sampling the biological material. A prerequisite is the confidentiality of the information received. After communication between the researcher and the patient, in the case of the voluntary consent of the latter, a questionnaire should be signed in the presence of a third disinterested person, who also signs the questionnaire.

The sampling of the biological material is made in the manipulation room to a special laboratory vessel, which is marked by the staff, and the patient's surname and initials are encoded by the envisaged coding of the study in order to preserve confidentiality. Further, only the sample number and the study code are used.

Ethically complicated issues arising in the study of single-nucleotide gene polymorphisms of work-related diseases distinguish the ethics of medical genetics from some other sections of bioethics. What are the most frequently asked questions during our research?

- how to properly diagnose a work-related disease, so that only a patient should be aware of the results and could not disseminate the information himself (to another research participant or employer)?
- what if a work-related illness is already o its late stages and its reverse development is not possible, and it is found that a patient has an individual high risk of developing is disease?
- how to properly work with the biological material: isolate, study, transform, etc.?
- how to use genetic information: store, transfer, distribute, destroy?

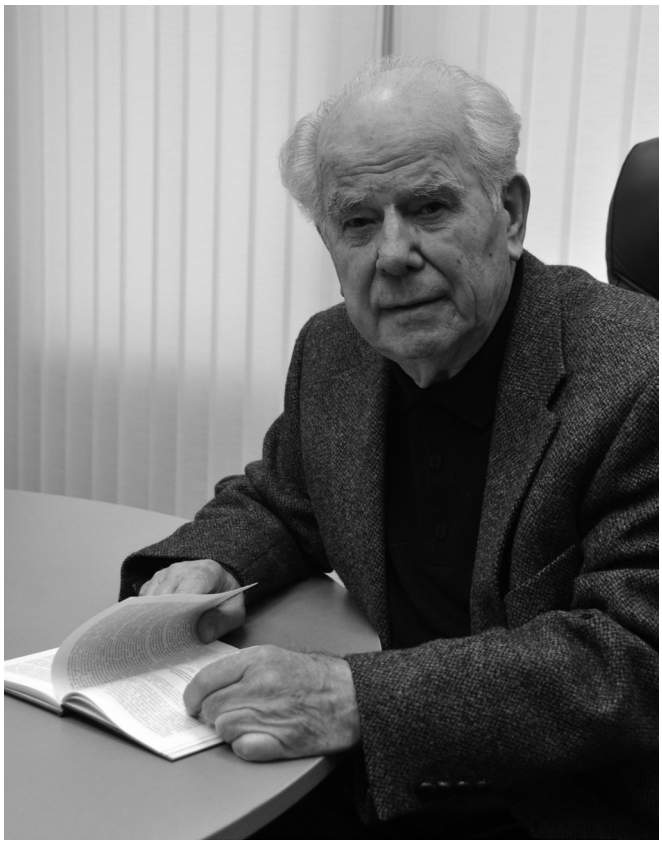

Fig. 1. Leader and founder of the bioethical movement in Ukraine, Academician Yuriy Kundiiev.

In 1997, at the 29th session of UNESCO, the "Universal Declaration on Genome and Human Rights" was adopted. Among its basic tenets of ethical principles of medical genetics our attention was focused on the following principles:

- consent to participate in medical-genetic procedures, testing and treatment;
- respect for a person's personality, regardless the level of his/her knowledge;
- close cooperation with organizations that unite patients and their relatives;
- use of understandable accessible language when communicating with a patient;
- constant monitoring of the quality of genetic services and procedures;
- prevention of discrimination in employment with insurance or training based on genetic information. Among the ethical principles of medical genetics, the latter deserves special attention. It states that the results of sin-

gle-nucleotide gene polymorphisms associated with the risk of developing an occupational disease should in no case be made known to an employer or other interested person, but only to the patient. If a patient is found to have an individual inherited tendency associated with an increased risk of developing a certain MFD, then he or she should be informed of the risks involved and should not be restricted in his or her choice of profession. If a person is only about to enter a profession with hazardous and dangerous working conditions, then he or she should be provided with comprehensive information about the high risks of certain occupational diseases associated with his or her future employment. What if a patient already has a certain length of service in harmful working conditions and at the same time it is found out that at the same time he has an individual high risk of developing a certain work-related MFD? The answer is one… only compelling medical advice and clarification in a readily available language about medical contraindications, high risks of developing MFD that are individually determined with the patient's voluntary consent. An explanation of rational employment is also needed to allow the patient to prolong duration and quality of life.

CONCLUSIONS

Therefore, single-nucleotide gene polymorphisms are latent life-long risk factors that can be developed under conditions of exposure to harmful environmental factors to a pathological process. Genetic factors cannot be modified, and there is a unique opportunity to use fundamentally new ways of preventing occupational MFD based on the consideration of individual inherited-related characteristics of the working person. Mass genotyping and formation of risk groups in occupational groups with subsequent survey, based on individual genetic predisposition is a cost-effective method of preventing work-related diseases. Complicated bioethical issues that arise when studying single-nucleotide gene polymorphisms of occupational diseases need to be actively discussed, not only by physicians, occupational pathologists, employers, scientists, but also by politicians and lawyers, taking into account ethical and social norms and implications.

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REVIEW ARTICLE
PRACA POGLĄDOWA

CONSTITUTIONAL AND ADMINISTRATIVE ASPECTS OF THE UKRAINE'S MEDICAL CODE

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ABSTRACT

The aim of this study is to show which aspects of Ukraine's constitutional and administrative law should appear in Ukraine's future medical code.

Materials and methods: The authors analyse five pieces of law or proposed law, including the 1996 Constitution's provisions on health care, *Law the Fundamentals of Health Protection* and the main codes. The authors apply classical legal analysis to these laws – analyzing the first three chapters of the proposed medical code from a constitutional and administrative perspective. The other methods used by the authors are systemic, comparative and synergetic.

Conclusion: Ukraine needs a medical code incorporating international and European health care standards. Such a code will also further develop the country's medical legislation. Yet the proposed project has many constitutional and administrative weaknesses.

KEY WORDS: health care reform, medical code, public health, right to the medical assistance, right to the health care

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INTRODUCTION

At present, the uncodified Ukrainian medical legislation's main drawback consists of its lack of constitutional and administrative basis. The country's parliament – the Verhovnya Rada – has yet to ratify the bill reforming the law Fundamentals of Health Protection[1].

The government has adopted several previous laws. Some of the most recent examples include the 2005 President's Decree On Urgent Measures to Reform the Public Health System, 2007 National Plan for the Development of the Health Care System for the Period up to 2010, Plan of Law-Making Process for 2008, On Approval of the Program of Activities: Ukrainian Breakthrough: For People, Not Politicians (all approved by the Cabinet of Ministers). Therefore, the government has not revised or reformed – for a long time – regulations and administrative acts leading to the Medical Code's development.

THE AIM

The aim of this study is to show which aspects of Ukraine's constitutional and administrative law should appear in Ukraine's future medical code.

MATERIALS AND METHODS

The main materials of the research are the norms of the Constitution of Ukraine of 1996 concerning healthcare and those of interpretation, the decisions of the Constitutional Court of Ukraine, the Civil Code of Ukraine, the draft Medical code of Ukraine – draft law "On Amendments to the Fundamentals of legislation on healthcare", the Concept of reform of financing the healthcare system, the Concept of development of the system of public health, the Code of Ukraine on Administrative Offences.

In the analysis of the norms of the Fundamental Law of Ukraine the authors used an axiological approach and primarily the systemic method to cover the norms of the first two sections, that concern protection of health; at that of importance were also the methods of analysis and synthesis. The technical legal and comparative methods have been used in comparison of norms of medical and administrative legislation of Ukraine, and the synergetic method enabled a new look at the state regulation of healthcare and the problem of elimination of controversies in normative and other legal acts.

REVIEW AND DISCUSSIONS

Ukrainian legal authors research mostly the civil law aspects of the proposed medical code. Experts propose to codify all of the civil law provisions related to health care [1]. Some local lawyers pay the utmost attention to the codification of Ukraine's civil procedural articles [2]. Researchers propose to modernize Soviet inspired health care and medical policy, using the civil law and Civil Code as the means – as legislators codified Ukraine's modern civil law after independence. They did not choose to use admin-

[1] The lawmakers refer to it as to the Medical Code, in this article the authors refer to it as to the 'proposed medical code'.

istrative law reform, as Ukraine's Administrative Code still dates from Soviet times [3]. Some authors though, argue that international law, not the civil law, should provide the model for reform—as Ukraine has not ratified many international standards into its domestic legislation [4]. Textbooks in Ukrainian medical law follow current civil law norms, with teaching and instructional cases coming mostly from the civil law [5].

However, in these works, authors pay almost no attention to constitutional and administrative aspects of the proposed medical code. These experts in constitutional and administrative law almost do not research the constitutional and jurisprudential problems involved in codifying Ukraine's medical law. Saribayeva represents the exception, with her works looking at methods and consequences of systematizing the country's health care law [6]. This study shows which aspects of Ukraine's constitutional and administrative law should appear in Ukraine's future medical code.

As of the moment, the organization of healthcare system of Ukraine is incapable of guaranteeing the right of the citizens to quality medical assistance, therefore, the primary task of the Medical code of Ukraine is creation of legal foundations for state regulation of protection of public health, that would encompass not only provision of medical services, but also the system of environmental safety and improvement of social environment in the country. Therefore, it is necessary: first, to introduce necessary corrections in the names of sections, chapters and articles of the Medical code; secondly, to specify not only the powers of the bodies of state government and local self-government in the sphere of public health, but also their duties on guaranteeing the rights of the interested public in this sphere; thirdly – exclude the provisions on the procedure of licensing the medical activities, limiting them to indication of obligatoriness of licensing of certain types of such activity; fourth – improve the norms on procedure and conditions of application of influence methods, as long as they are the measures of civil, criminal, administrative responsibility which are applied in accordance with the procedure prescribed by law in case of a violation of the legislation on public healthcare.

1. The Constitutional Provisions of the Proposed Ukrainian Medical Code

Ukraine's constitution's authors created the foundation for the regulation of health issues in the country. The constitution guarantees the right to health care, medical care and medical insurance (art. 49). The supreme law also declares the country as a "social state" (art. 1). Moreover, the constitution provides that, "the person, his/her life and health, honor and dignity, inviolability and security are recognized in Ukraine as the highest social value. Human rights and freedoms and their guarantees determine the content and direction of the state's activities. The state is responsible to a person for his activities. The assertion and guarantee of human rights and freedoms is the main responsibility of the state" (art.3) [7].

In addition, a number of the 1996 Constitution's articles allow the government to restrict human rights in the interests of public health. Examples include freedom of opinion and religion (art.35), the right to freedom of association in political parties and public organizations (art.36), the right to gather peacefully, without weapons and hold meetings and demonstrations (art.39).

The proposed medical code claims that health care legislation requires that "it [the legislation] is based on the Constitution of Ukraine" (art. 1). The proposed medical code further posits that, "Health care is the duty of the state, citizens and society" (the title of art. 2). The article further provides that, state bodies, local government, enterprises, institutions and organizations of all forms of ownership [as well as] officials, citizens and their associations are obliged to ensure the priority of health care in their own activities; not to harm the health of the population and individuals, to provide assistance to patients, disabled persons, victims of accidents and in the event of an emergency within the limits of their competence, to assist employees of health facilities in their work and perform other duties provided by law [8].

This article adequately reflects the transition from the Semashko model. This model envisaged full state financing of health care. The proposed medical code seeks to promote each person's own financial responsibility (with contributions made by the state) for his or her own health. Lawmakers wrote the article conforming to the two related constitutional court decisions on the health care. These decisions comprise the 1998 *Decision on Case of the Paid Medical Services* and the 2002 *Decision on Free Medical Assistance* [9, 10].

Lawmakers tried to expand on the Constitution's art.49 right to health care in chapter 3 of the proposed medical code, covering the basics of citizen's right to health care (art. 7), the rights of foreigners and stateless persons to health care (art. 8), guarantees of the right to health care (art. 9), restrictions on the citizens' health related rights (art. 10), citizens' health related responsibilities (art. 11). But problems remain with the proposed medical code. Firstly, articles 7-11 of the draft law are very short and lack details. Secondly, the Civil Code already explained the constitutional right to health care in articles 201, 270, 283-286 [8]. Lawmakers should avoid duplication in the Civil Code and the Medical Code. They should expand on the Constitution's Article 49 provisions only in the Civil Code.

2. Administrative Provisions of the Proposed Ukrainian Medical Code

Article 49 of the Constitution not only declares the right to health services and medical assistance for every person, but also obligates the state to create conditions for effective and accessible medical services for all citizens. However, at present the organization of the health care system not only violates a person's constitutional right to accessible and high-quality medical care. The system also negatively affects Ukrainians' health and life expectancy. Furthermore, such an organisation hinders economic development and social solidarity [10-11]. The health care system therefore needs urgent reform.

The state should take the leading role in this reform. The supreme law validates the proposed medical code's

importance for this reform, as 'the legal foundations for health care regulation are determined exclusively by the laws of Ukraine' (art. 92).

The successful reform, based on codifying existing medical legislation, depends on effective change management. The proposed medical code describes state health care regulation. In contrast to state governance of health care activities, state *regulation* encompasses a broader set of issues. Such a regulatory effect expands to the entire field of health care in the country. Such a field includes, according to Article 11 of the draft code, the entirety of health care institutions, as well as medical and other professionals, who work at those institutions or individuals, providing preventative medical services. The article also defines such a field to include bodies managing health care, which ensures direct state control, regulation and inter-sectoral participation in this specified field, as well as in the broader society.

The proposed medical code describes state health care regulation, aimed at enforcing a unified and effective state health care policy; the protection of the interests of the citizens who receive medical care, the creation of favorable conditions for the development and functioning of health care institutions under all forms of ownership and all organizational forms, ensuring opportunities for equal access to medical assistance for all social groups, the prevention of monopolization and the creation of conditions for fair competition in the health care system, ensuring transparency and openness in the health care system, as well all supporting integration into European and global health care systems.

These goals can be grouped into three blocks – state health care policy-related goals, goals related to improving health care, and goals related to the development of the health care system. Lawmakers should reformulate Article 12 of the *Law the Fundamentals of Health Protection*. The aims of state health care regulation should consist of: 1) running effective health care policy, 2) securing citizens' rights to health care and, 3) creating an effective system of health care.

Lawmakers of the proposed medical code should consider not only the norms of 1996 Constitution, but also the administrative provisions, including the Cabinet of Ministers' documents on public health. For now, the proposed medical code mentions that the public health is an essential goal for the country's health care system functioning – all organizations, institutions and resources, intended for provision of any type of service, aimed at strengthening, renewal and support of health at individual or collective level, including through intersectoral cooperation. According to the proposed medical code's provisions, 'the system of public health is a set of instruments, procedures and measures implemented by state bodies and non-governmental institutions that strengthen individual and public health, prevent the diseases, increase the life expectancy, active and productive living, encourage the healthy lifestyle through coordination the entire society's efforts'[2].

Government has to improve the administrative regulation of the state health care system using various means, including the legislative provisions on public health, their monitoring and forecasting of future actions.

It appears necessary to introduce corrections in the names of the corresponding sections, chapters and articles of the Medical code. It concerns the state policy in the sphere of protection of public health, organization of protection of public health and state regulation in this sphere in Ukraine.

The provisions concerning protection of public health and state regulation in the field can be found in section II of the Medical code. Their implementation may ensure harmonious development of physical and spiritual well-being, high productivity, long and active life of the citizens, removal of factors that negatively affect their health, reduction of morbidity rate, disability and mortality. This section is of declaratory character, is cumbersome and many of its provisions are duplicative.

Article 2 of the proposed medical code proclaims that protection of public health is a general duty of the state, citizens and society, and the state bodies and bodies of local self-government, enterprises, institutions and organizations of all forms of property, their officials (employees), citizens and groups of citizens are obliged to ensure the priority of health care in their activities.

Article 4 declares the main principles of functioning of the system of protection of public health in Ukraine, which include ensuring of effective health care as a priority of the state activities in the sphere of public services, patient needs-oriented approach, priority of prophylactic activities, ensuring quality medical help through standardization of medical practices, which are the general principles of the legislation o Ukraine in the sphere of protection of public health, and not only functioning of the system of health care. As regards the principles of equality of citizens, information openness, decentralization of power, legality, ensuring human and citizen's rights and enforcement of related social guarantees, these are constitutional principles that do not require duplication in the Medical code of Ukraine.

Article 13 of the Draft determines the forms of state regulation of protection of public health (licensing, control and monitoring, application of influence mechanisms, standardization, etc.), which are administrative legal methods, the application of which is regulated by the corresponding norms of administrative law and carried out by the executive bodies. Chapter 7 of the Draft "Licensing of activities in the sphere of health care" contains provisions on obligatory licensing of certain types of medical activity, list of documents necessary to receive a license, procedure of adopting a decision or rejection in issuing a license. It

[2] Such approach fully corresponds to the WHO guidelines, which combine traditional criteria of health – physical and mental, with the lifestyle of a person determined by social, economic and environmental circumstances.

must be indicated that the corresponding relations are regulated by the Law of Ukraine "On licensing of certain types of commercial activities", Licensing conditions of commercial activities on medical practice, affirmed by the decree of the Cabinet of Ministers of Ukraine of 2 March 2016 No 285, therefore it is unnecessary to fully regulate the matter by the Medical code, thus Articles 29-32 should be excluded from the proposed medical code, and Article 28 should be formulated as follows:

Article 28. Obligatory licensing

1. The authorized body shall under the procedure specified by the legislation of Ukraine and within the limits of its competence issue licenses to the economic entities for commercial activities on:

1) medical practice;
2) production of medical supplies, wholesale and retail sale of medical supplies;
3) development, production, manufacturing, storing, transportation, acquiring, sending, import, export, sale and destruction of narcotic substances, psychotropic substances and precursors.

This concerns also the provisions on influence measures. Articles 34-39 should be excluded from the proposed medical code, and Article 33 should be formulated as follows:

Article 33. Responsibility for violation of legislation on protection of public health.

The violation of legislation on protection of public health entails disciplinary, administrative, civil and criminal responsibility in accordance with the legislation of Ukraine.

The annulment of license to carrying out the activities on provision of medical services, removal of leadership from managing a medical institution, are measures of administrative responsibility foreseen by Article 30 of the Code of Ukraine on administrative offenses – "Deprivation of a special right granted to a citizen, deprivation of right to occupy certain positions or carry out certain activities".

It must be noted that except legal responsibility, the offenders may be affected by measures of influence in the form of administrative enforcement – temporary (till removal of offences) suspension of activities.

It must also be stated that Article 14 of the proposed medical code "Bodies that carry out state policy in the sphere of health care – management, regulation and coordination" has an incorrect name, as long as there is a state policy introduced in the sphere of protection of public health. Except for that, it is generally recognized that the state policy is the activity of the bodies of state power and management aimed at solution of social problems, achievement and realization of sustainable development of the society and its certain spheres, among which is the sphere of public health, while regulation and coordination are the functions of public administration, therefore, the name of the Article 14 should be formulated as follows: "Realization of state policy in the sphere of protection of public health".

Alongside with that, it appears that inclusion of Chapter 5 if fully justified, as it describes the powers of the executive bodies – of the authorized body and other central bodies of executive power and organs of local self-government in the sphere of public health care, their tasks and organization of activities, as well as Chapter 46 – "Participation of the public in healthcare management". Alongside with that, the duties of state bodies in the sphere of ensuring the rights of the public, including the duty to provide information at requests of interested natural and legal persons, needs a more detailed formulation.

CONCLUSIONS

The authors conclude that Ukraine needs a medical code incorporating international and European health care standards. Such a code will also further develop the country's medical legislation. Yet the proposed project has many constitutional and administrative weaknesses.

Lawmakers begin this document with the provisions that prove: health of each person in particular and public health in general are amongst the state's main values. Furthermore, the lawmakers attempted to specify the constitutional right to health protection. However, they have not taken into account the Civil Code, which already does it. Thus, the articles of the proposed medical code mostly duplicate it. As the articles of the proposed medical code do not contribute to specify the constitutional right to health care, it is advisable to exclude them from this document.

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CASE REPORT
OPIS PRZYPADKU

MANIFESTACJE PŁUCNE CHOROBY LEŚNIOWSKIEGO-CROHNA CZY POWIKŁANIA PRZEWLEKŁEJ FARMAKOTERAPII? – OPIS PRZYPADKU

PULMONARY MANIFESTATIONS OF CROHN'S DISEASE OR CHRONIC PHARMACOTHERAPY COMPLICATIONS? – CASE REPORT

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STRESZCZENIE

Manifestacje pozajelitowe choroby Leśniowskiego-Crohna (ChLC), poza najczęstszymi manifestacjami skórnymi i stawowymi, obejmują także powikłania ze strony układu oddechowego. Również przewlekła farmakoterapia ChLC, zwłaszcza pochodnymi kwasu 5-aminosalicylowego czy lekami anty-TNF-α wiąże się z możliwymi pulmonologicznymi działaniami niepożądanymi, czasem o trudnym do zróżnicowania obrazie klinicznym. W prezentowanej pracy opisujemy chorą na ChLC, z wywiadem pneumocystozowego zapalenia płuc, u której zdiagnozowano złuszczające śródmiąższowe zapalenie płuc w wyniku przewlekłego stosowania mesalazyny. Choroba ta charakteryzuje się akumulacją pęcherzykowych makrofagów w światłach pęcherzyków płucnych i w przegrodach międzypęcherzykowych, a najczęstszym czynnikiem etiologicznym jest narażenie na dym tytoniowy. U naszej chorej, niepalącej, ostateczne rozpoznanie postawiono po biopsji płuca i ocenie histopatologicznej. Stopniowa poprawa kliniczna po odstawieniu mesalazyny była dodatkowym czynnikiem potwierdzającym etiologię choroby. Opisywane powikłanie nie jest częstym działaniem niepożądanym po mesalazynie, ale należy o nim pamiętać u wszystkich chorych leczonych preparatami kwasu 5-aminosalicylowego.

SŁOWA KLUCZOWE: choroba Leśniowskiego-Crohna, mesalazyna, działania niepożądane, śródmiąższowe zapalenie płuc, manifestacje płucne

ABSTRACT

Pareneteral manifestations of Crohn's disease (ChLC), apart from the most common skin and joint symptoms include also complications from the respiratory system. In addition chronic pharmacotherapy of ChLC, especially 5-aminosalicylic acid or anti-TNF- α drugs, is associated with possible pulmonologic side effects, sometimes difficult to differentiate. In this study, we describe a patient with ChLC, with a history of pneumocystic pneumonia, who was diagnosed with exfoliative institial pneumonitis as a result of chronic use of mesalazine. This disease is characterized by accumulation of alveolar macrophages in the lumen of the alveoli and intrabepticular septum. The most common etiologic factor is exposure to tobacco smoke. Our patient, non-smoker, was finally diagnosed after lung biopsy and histopathological evaluation. The gradual clinical improvement after mesalazine was an additional factor confirming the etiology of the disease. This side effect of mesalazine is not common but it should be considered in all patient treated with 5-aminosalicylic acid.

KEY WORDS: Crohn's disease, mesalazine, side effects, pneumocistits pneumonia, pulmonary manifestations

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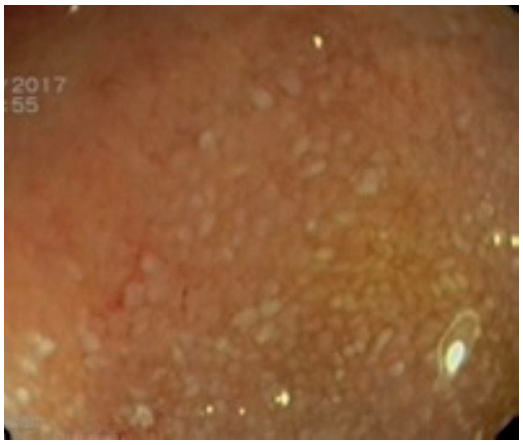
WSTĘP

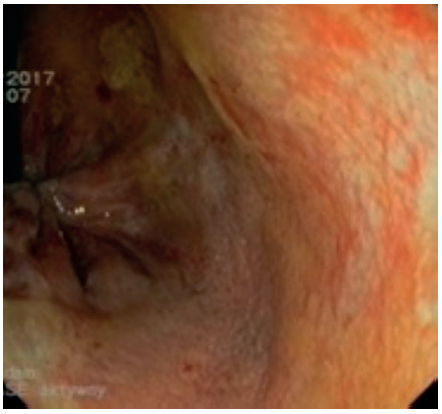
Choroba Leśniowskiego-Crohna (ChLC) jest pełnościennym, nieswoistym procesem zapalnym mogącym obejmować każdy odcinek przewodu pokarmowego. Częstość jej występowania stale rośnie we wszystkich regionach świata i jest wyższa w krajach rozwiniętych aniżeli rozwijających się, oraz na terenach zurbanizowanych w porównaniu z terenami wiejskimi [1]. We wszystkich regionach słabo rozwiniętych wraz z ich rozwojem socjoekonomicznym zauważa się stały wzrost częstości występowania i rocznej zapadalności na ChLC [2, 3].

Dla nieswoistych chorób zapalnych jelit charakterystyczne są manifestacje pozajelitowe, najczęściej skórne, oczne i stawowe. Rzadziej obserwowane, ale o poważnych konsekwencjach klinicznych są powikłania ze strony układu oddechowego. Przykłady takich manifestacji stanowią choroby miąższu płuc, takie jak: ostre śródmiąższowe zapalenie płuc i samoorganizujące się zapalenie płuc, a także choroba zakrzepowo-zatorowa, choroby opłucnej oraz ostre zapalenie i nadreaktywność oskrzeli [13]. Również przewlekła farmakoterapia ChLC, zwłaszcza pochodnymi kwasu 5-aminosalicylowego (5-ASA) czy anty-TNF-α wiąże się z możliwymi powikłaniami ze strony układu oddechowego, czasem o trudnym do zróżnicowania obrazie klinicznym. Najpowszechniej stosowaną pochodną kwasu

Ryc. 1. Kolonoskopia wykonana 14 lutego 2017 roku.
Błona śluzowa zstępnicy z zatartym rysunkiem naczyniowym i z krwawieniem kontaktowym
Zdjęcia: Pracownia Endoskopowa, Klinika Chorób Przewodu Pokarmowego USK nr 1 im. N. Barlickiego w Łodzi



Ryc. 2. Kolonoskopia wykonana 14 lutego 2017 roku.
Błona śluzowa esicy z zatartym rysunku naczyniowym, obrzęknięta, pokryta włóknikiem.
Zdjęcia: Pracownia Endoskopowa, Klinika Chorób Przewodu Pokarmowego USK nr 1 im. N. Barlickiego w Łodzi.



Ryc. 3. Kolonoskopia wykonana 14 lutego 2017 roku.
Błona śluzowa odbytnicy o zachowanym rysunku naczyniowym, gładka, w rektoskopii widoczne guzki krwawnicze odbytu.
Zdjęcia: Pracownia Endoskopowa, Klinika Chorób Przewodu Pokarmowego USK nr 1 im. N. Barlickiego w Łodzi.

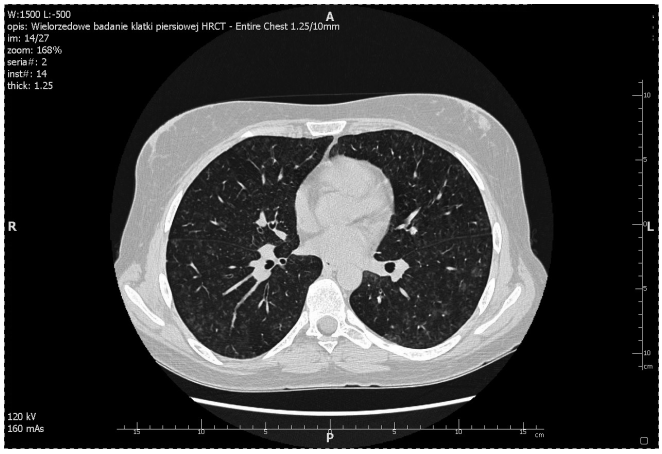
5-aminosalicylowego jest mesalazyna, stosowana zarówno w leczeniu nieswoistych chorób zapalnych jelit, jak i jako chemoprewencja raka jelita grubego. Najczęściej jest ona lekiem dobrze tolerowanym, ale możliwe są rzadko obserwowane poważne powikłania płucne takie jak eozynofilowe zapalenie płuc, samoorganizujące się zapalenie płuc, czy niespecyficzne śródmiąższowe zapalenie płuc [4, 5].

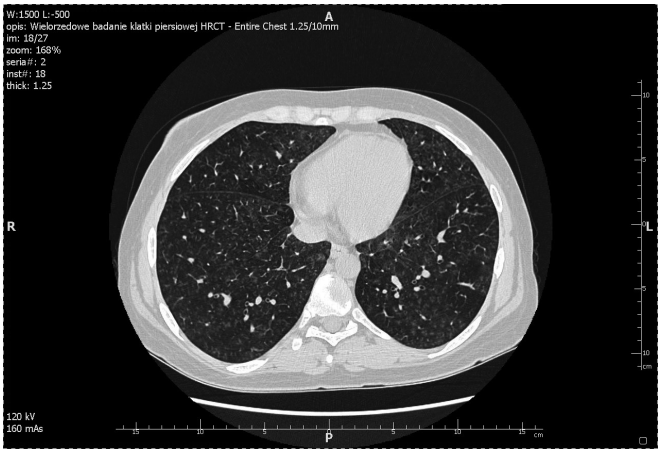
OPIS PRZYPADKU

Obecnie 28-letnia chora, z wywiadem choroby Hashimoto, atopowego zapalenia skóry i wieloważnej alergii powietrznopochodnej zgłosiła się do szpitala w 2016 roku z powodu bólów brzucha i biegunek do 5 razy na dobę, bez domieszek patologicznych. W wykonanej kolonoskopii uwidoczniono zwężenie poprzecznicy, nieprzepuszczające aparatu, o zaczerwienionych brzegach i ze zlewającymi się owrzodzeniami, które widoczne były również w zstępnicy i esicy. W wykonanej tomografii komputerowej (TK) jamy brzusznej opisano dodatkowo zwężony początkowy odcinek dwunastnicy i wąskie światło pogrubiałej pętli jelita czczego. Wysunięto podejrzenie ChLC, chora otrzymała steroidoterapię, leczenie żywieniowe oraz preparaty 5-ASA – mesalazynę.

Po krótkiej poprawie w styczniu 2017 roku wystąpiło kolejne zaostrzenie choroby i pogorszenie ogólnego stanu pacjentki, z niedokrwistością (HGB 9,2 g/Dl), gorączką i stanem zapalnym (CRP 129,7 mg/l). Chora została hospitalizowana w Klinice Chorób Przewodu Pokarmowego Uniwersytetu Medycznego (UM) w Łodzi. W wykonanej pełnej kolonoskopii błona śluzowa jelita grubego obrzęknięta, pokryta włóknikiem, z zatartym rysunkiem naczyniowym, licznymi wybroczynami i krwawieniem kontaktowym na całym obejrzanym odcinku jelita za wyjątkiem odbytnicy (Ryc. 1–3). W dodatkowo przeprowadzonej enteroskopii dwubalonowej uwidoczniono wygładzone fałdy okrężne w początkowym odcinku jelita cienkiego, bez zwężeń i innych patologii. Zadecydowano o dodatkowym włączeniu azatiopryny, utrzymując mesalazynę i steroidoterapię w zmniejszanych dawkach. Trzy miesiące później, w kwietniu 2017 roku, u pacjentki pojawiły się: gorączka, suchy kaszel i duszność, a dodatkowo w badaniach laboratoryjnych obserwowano istotną leukopenię do 1,4 tys/mm^3. Zdjęcie radiologiczne oraz TK klatki piersiowej wykazały masywne zagęszczenia miąższowe obu płuc. Odstawiono azatioprynę, a z posiewu krwi wyhodowano bakterie *Klebsiella pneumoniea ESBL* – zastosowano celowaną antybiotykoterapię, bez poprawy klinicznej. Chora została przeniesiona do Instytutu Gruźlicy w Warszawie, gdzie w wykonanej bronchoskopii z biopsją potwierdzono pneumocystozowe zapalenie płuc z towarzyszącym zapaleniem gronkowcem złocistym opornym na metycylinę (MRSA). Gruźlicę ostatecznie wykluczono, a po zastosowaniu kotrimoksazolu nastąpiła poprawa stanu klinicznego chorej. Wyleczono pneumocystozowe zapalenie płuc oraz zalecono przedłużone profilaktyczne leczenie kotrimoksazolem podczas leczenia immunosupresyjnego.

W czerwcu 2017 roku, przy próbie zmniejszenia dawki sterydów doszło do ponownego nasilenia objawów ze strony





Ryc. 4, 5. Badanie HRCT klatki piersiowej wykonane 14 sierpnia 2018 roku w Uniwersyteckim Szpitalu Klinicznym im. N. Barlickiego w Łodzi - widoczne zmiany o charakterze mlecznej szyby w obu polach płuc.

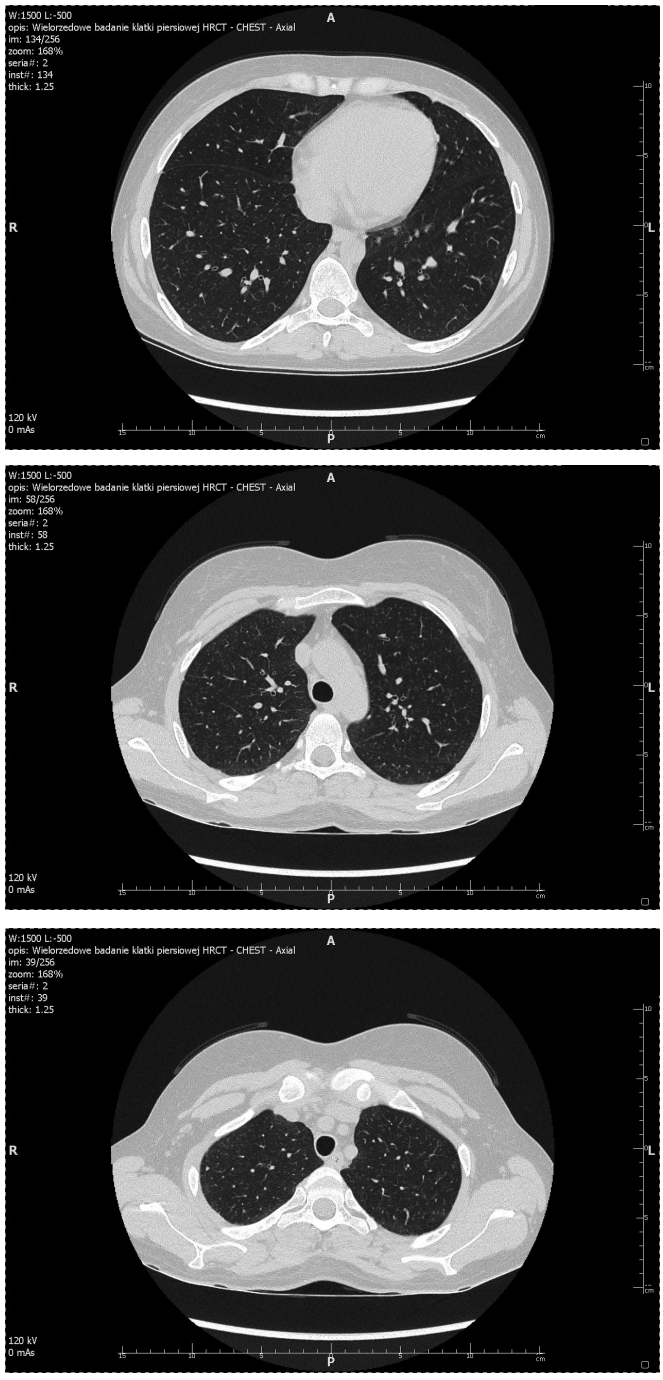
układu pokarmowego, ze względu na steroidozależność zadecydowano o włączeniu terapii biologicznej. Pacjentka była leczona adalimumabem, z dobrą odpowiedzią kliniczną i z uzyskaniem remisji po 3 miesiącach terapii. Kontynuowano leczenie podtrzymujące adalimumabem w skojarzeniu z mesalazyną do 12 miesięcy zgodnie z programem terapeutycznym NFZ.

Po zakończeniu terapii biologicznej, w lipcu 2018 roku, pacjentka zgłosiła zaburzenia równowagi, zawroty głowy i była hospitalizowana w Klinice Neurologii UM w Łodzi. Dodatkowo zgłaszała duszność, suchy kaszel, męczliwość i osłabienie. W wykonanych badaniach obrazowych głowy nie wykryto organicznej przyczyny zaburzeń równowagi. Chora została przeniesiona do Kliniki Pulmonologii i Alergologii UM, gdzie w TK klatki piersiowej uwidoczniono liczne rozsiane śródzrazikowe guzki z towarzyszącym obrazem mlecznej szyby w prawym i lewym płucu (Ryc. 4-5). Pacjentce zaproponowano odstawienie mesalazyny oraz leczenie sterydami, jednak nie wyraziła zgody na stosowanie sterydów ze względu na występujące wcześniej powikłania po sterydoterapii. Wykonano spirometrię, dokumentując obturację w stopniu umiarkowanym z FEV1 – 48%, bez zaburzeń w badaniu zdolności dyfuzyjnej płuc dla tlenku węgla (DLCO) oraz bronchoskopię, w której nie uwidoczniono nieprawidłowości. Wykonano badanie popłuczyn oskrzelowo-pęcherzykowych, w analizowanym materiale limfocyty stanowiły 60,0%, a eozynofile 3,0% komórek. Wysunięto podejrzenie śródmiąższowej choroby płuc w przebiegu leczenia mesalazyną, która została odstawiono.

Ze względu na wątpliwości diagnostyczne wykonano biopsję płuca w Klinice Torakochirurgii UM w Łodzi. W badaniu histopatologicznym opisano fragment miąższu płuca o ogniskowo zaburzonej architektonice, a w licznych pęcherzykach i oskrzelikach płucnych nacieki zapalne z limfocytów i komórek histiocytarnych. Dodatkowo uwagę zwracało wyraźne pogrubienie ścian naczyń krwionośnych, a w świetle oskrzelików obecne bezpostaciowe kwasochłonne masy. Postawiono podejrzenie złuszczającego śródmiąższowego zapalenia płuc. Podtrzymano decyzję o odstawieniu mesalazyny, stan kliniczny chorej poprawiał się, a w kontrolnej spirometrii we wrześniu 2018 roku nastąpiła także normalizacja parametrów oddechowych (Ryc. 6–8). Aktualnie pacjentka nie przyjmuje mesalazyny, a chora jest w remisji klinicznej.

DYSKUSJA

Częstość występowania manifestacji płucnych w przebiegu NChZJ wynosi od 20 do nawet 40% [7, 12, 14]. Rzadziej dochodzi do powikłań płucnych po stosowanej farmakoterapii, najczęściej o charakterze infekcyjnym, ale także z powodu nadwrażliwości na stosowane leki. Najczęstsze opisywane działania niepożądane i powikłania ze strony układu oddechowego po mesalazynie to: suchy kaszel, duszność, ostra niewydolność oddechowa, hypoksemia, a także ból w klatce piersiowej pochodzenia opłucnowego [10].
U naszej chorej obserwowaliśmy bardzo rzadkie płucne działania niepożądane prawdopodobnie po zastosowaniu mesalazyny pod postacią złuszczającego śródmiąższowego zapalenia płuc. Niewiele jest podobnych przypadków chorych opisywanych w piśmiennictwie, w tym wywołane farmakoterapią zarostowe zapalenie oskrzelików z organizującym się zapaleniem płuc i włóknienie płuc [8, 10, 15]. Złuszczające śródmiąższowe zapalenie płuc zaliczane jest do tzw. grupy chorób wywoływanych przez palenie (SR- ILD – *Smoking-Related Interstitial Lung Disease*). Palenie papierosów jest przyczyną choroby w 58–91%, dużo rzadziej może być ona konsekwencją przewlekłego stosowania preparatów kwasu 5-ASA, w tym sulfasalazyny lub mesalazyny [11]. Złuszczające śródmiąższowe zapalenie płuc charakteryzuje się akumulacją pęcherzykowych makrofagów w światłach pęcherzyków i przegrodach. Choroba najczęściej rozwija się u osób w wieku 40–50 lat, wtórnie do aktywnej lub biernej ekspozycji na dym tytoniowy, nasza pacjentka jednak nigdy nie paliła papierosów, ani nie potwierdza biernego narażenia na dym tytoniowy [16]. W leczeniu stosuje się farmakoterapię złożoną z doustnych glikokortykosteroidów, ale wykorzystywana jest również azatiopryna. Dziesięcioletni czas przeżycia chorych na złuszczające śródmiąższowe zapalenie płuc oceniany jest



Ryc. 6, 7, 8. Zdjęcie wykonane 28 listopada 2018 roku. W porównaniu do poprzedniego badania HRCT z dnia 14 sierpnia 2018 roku stwierdza się niemal całkowitą regresję zmian śródmiąższowych - obecnie słabo separują się mniej liczne, drobne guzki śródmiąższowe o obrazie matowej szyby głównie w segmentach 1, 2, 1+2 i 3 płatów górnych obu płuc. Niecharakterystyczne guzki w segmentach 1P, 2P i 1+2P wielkości około 3 mm, w segmentach 6 obustronnie wielkości do 5mm. Struktury śródpiersia w zakresie dostępnym ocenie w badaniu HRCT bez zmian ogniskowych, wnęki obustronnie poszerzone jak w badaniu poprzednim, raczej węzłowo, poza tym śródpiersie bez cech adenopatii.

na 70–88%, a jej śmiertelność waha się od 6 do 28% [11]. Złuszczające śródmiąższowe zapalenie płuc jako powikłanie nieswoistych chorób zapalnych jelit, w tym ChLC, opisywane jest niezwykle rzadko, właśnie jako powikłanie stosowania leków z grupy 5-ASA [17]. Potwierdzeniem rozpoznania jest wynik badania histopatologicznego po biopsji płuca, a dodatkowo ustala je poprawa kliniczna po odstawieniu prawdopodobnego czynnika etiologicznego.

U opisywanej chorej dodatkowym czynnikiem obciążającym była 12-miesięczna terapia adalimumabem, przeciwciałem przeciwko TNF-α, po którym także opisywano występowanie śródmiąższowych zmian zapalnych w płucach [18]. Patogeneza chorób płuc indukowanych stosowaniem leczenia anty-TNF-α pozostaje niewyjaśniona. Jedną z możliwych hipotez jest fibrogenne działanie na tkankę płucną poprzez stymulowanie wytwarzania kolagenu przez fibroblasty i miofibroblasty. Ponadto zwiększa się intensywność odpowiedzi limfocytów Th2 i wzrasta aktywność INF-gamma, co ma wpływ na prozapalne zmiany w tkance płucnej [19]. Dodatkowo u naszej chorej w przeszłości wystąpiło zapalenie płuc o charakterze grzybiczym, wywołane przez *Pneumocystis jiroveci*. Przed okresem wczesnego wykrywania zakażenia ludzkim wirusem niedoboru odporności (HIV), *Pneumocystis jiroveci* było wczesnym wskaźnikiem występowania zakażenia HIV. Częstość występowania *Pneumocystis jiroveci* u chorych zakażonych wirusem HIV zaczęła spadać po wprowadzeniu skutecznych leków antyretrowirusowych. Aktualnie zapadalność na *Pneumocystis jiroveci* wzrasta u chorych onkologicznych, po przeszczepach narządów czy przyjmujących immunosupresję z innych wskazań medycznych. Objawy choroby są zazwyczaj niespecyficzne, obejmują gorączkę, suchy kaszel, trudności w oddychaniu czy ból w klatce piersiowej [20]. U opisywanej chorej czynnikiem ryzyka zakażenia *Pneumocystis jiroveci* były antybiotykoterapia, przewlekła steroidoterapia i leczenie immunosupresyjne w wywiadzie. W wywiadzie pacjentki występowała także choroba Hashimoto, która może objawiać się uczuciem zmęczenia, objawami ze strony układu pokarmowego, takimi jak: zaparcia, problemy trawienne, hipotonia pęcherzyka żółciowego, a także niedokrwistość [21]. Opisywana pacjentka pozostawała jednak w stanie eutyreozy – przewlekła farmakoterapia lewotyroksyną przynosiła oczekiwane efekty. W badaniach występowały prawidłowe wyniki TSH oraz prawidłowe wyniki poziomu hormonów tarczycy. W opinii pracowników Kliniki oraz konsultującego endokrynologa, prezentowane objawy takie jak zmęczenie nie wynikały z choroby tarczycy. Obraz manifestacji płucnych w przebiegu NChZJ może być bardzo zróżnicowany, a ustalenie czy są to manifestacje pozajelitowe ChLC, czy powikłania po stosowanej farmakoterapii bywa dużym wyzwaniem klinicznym [9]. NChZJ wymagają przewlekłego leczenia, w tym terapii immunosupresyjnej i biologicznej, które mogą dawać poważne działania niepożądane. Jednak opisywana przez nas chora rozwinęła ciężkie działania niepożądane po mesalazynie, czyli szeroko stosowanemu preparatowi 5-ASA, mającego opinię leku bezpiecznego i dobrze tolerowanego, zalecanego także jako chemoprewencja raka jelita grubego u chorych na NChZJ. Opisywane powikłanie nie jest częste, ale należy o nim pamiętać u wszystkich chorych leczonych preparatami kwasu 5-aminosalicylowego.

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CASE REPORT
OPIS PRZYPADKU

NON-ST-ELEVATION ACUTE CORONARY SYNDROME DUE TO A TOTALLY OCCLUDED CORONARY ARTERY: A HISTORY OF TWO TWIN BROTHERS

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ABSTRACT
We present the image of two twin brothers aged 53. Within 18 months they both underwent acute coronary syndrome treated with percutaneous coronary intervention (PCI). This story shows that both twins had similar comorbidities (hypercholesterolemia and hypothyroidism) as well as the course of the acute coronary syndrome. Although in both cases the coronary artery was totally occluded (in one case – fresh occlusion, in the other – CTO), no STEMI presentation was observed. Therefore, thorough investigation is warranted in twins after the acute coronary syndrome in of them, even in case of no evident ischemia symptoms.

KEY WORDS: genetic predisposition; hereditary; coronary artery disease

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INTRODUCTION
Genetic predisposition to the development of coronary artery disease and myocardial infarction is characterized enough. Nevertheless, the lack of replication, and problems in the recognition of specific genes still pose a challenge at the molecular level. It is mainly associated with the phenotype heterogeneity and underlying genotypes. Below we present the image of two twin brothers aged 53. Both had a history of hypothyroidism and hypercholesterolemia.

CASE REPORT
In April 2018 one of them presented with non-ST-elevation MI (NSTEMI). Coronary angiography revealed proximal occlusion of the left circumflex artery, and subsequent successful percutaneous coronary intervention (PCI) with drug-eluting stent (DES) was performed (Fig. 1A-C). In echo at discharge akinesis of lateral and posterior wall was shown with ejection fraction (EF) of 45%. The patient received aspirin, ticagrelor, nebivolol, ramipril, atorvastatin and l-thyroxine.

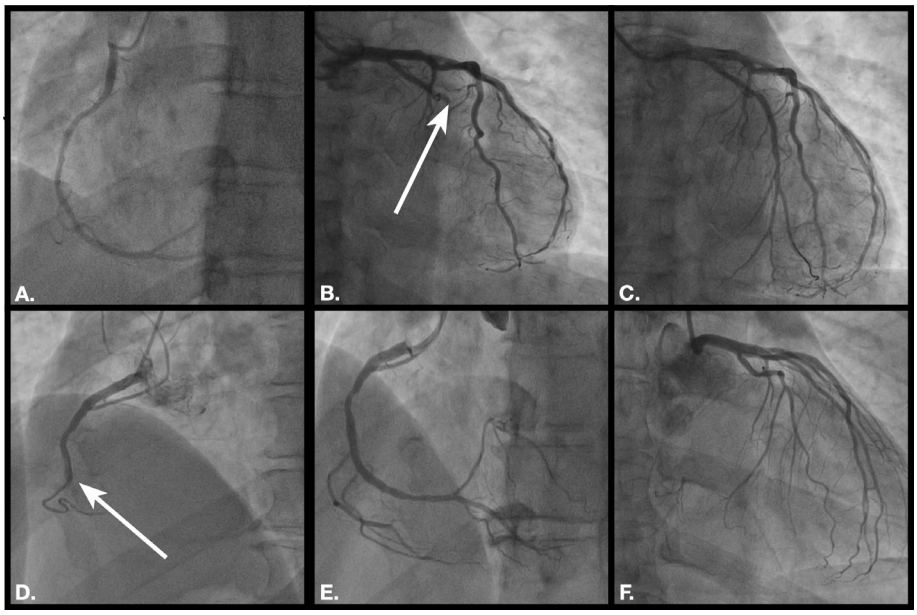

Fig. 1. Coronary angiography of two twin brothers. (A) Twin A – right coronary artery, (B) Twin A – left coronary artery with occluded left circumflex (white arrow), (C) Twin A – left coronary artery after successful percutaneous coronary intervention with drug-eluting stent implantation, (D) Twin B – right coronary artery occluded in the mid segment (white arrow), (E) Twin B – right coronary artery after successful percutaneous coronary intervention with drug-eluting stent implantation, (F) Twin B – left coronary artery.

One year and five months later a second brother during the exercise treadmill test developed a presyncope with a large drop in blood pressure. The patient was referred to the emergency department. Cardiac enzymes were negative, but taking into consideration the complete history invasive diagnostics was planned. Coronary angiography showed medial occlusion of the right coronary artery. Successful PCI with DES was performed in this chronic total occlusion (Fig. 1D-F). In echo hypokinesis of inferior wall was shown with EF of 53%. The patient received aspirin, clopidogrel, nebivolol, ramipril, atorvastatin and l-thyroxine.

This story shows that both twins had similar comorbidities (hypercholesterolemia and hypothyroidism) as well as the course of the acute coronary syndrome. Although in both cases the coronary artery was totally occluded (in one case – fresh occlusion, in the other – CTO), no STEMI presentation was observed. In previous studies it was unquestionably proved that genetic factors play the important role in the development of coronary artery disease or myocardial infarction [1, 2]. In particular, research studies on twins estimated the heritability of coronary artery disease and myocardial infarction to be approximately 50% to 60% [3]. Therefore, comprehension of genetic mechanisms for coronary artery atherosclerosis development will not only be of some use to understand the pathogenesis of the coronary artery disease, but may also be the foundation for creating specific prevention and treatment strategies [4].

CONCLUSIONS

In conclusion, this image shows that thorough investigation is warranted in twins after the acute coronary syndrome in of them, even in case of no evident ischemia symptoms.

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Conflict of interest
Authors declare no conflict of interest.

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CASE REPORT
OPIS PRZYPADKU

MULTIPLE MYELOMA: CHALLENGES OF DIFFERENTIAL DIAGNOSIS (CLINICAL CASE)

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ABSTRACT

The objective of our study was to interpret and discuss atypical multiple myeloma case. The article describes the case of clinical observation of a patient K, in which manifestations of chronic kidney disease and circulatory failure prevailed in clinical picture of the disease. The authors recommended an X-ray examination of skull and pelvic bones as a screening method suitable for elderly people with symptoms of chronic renal insufficiency and chronic bone and muscle pain resistant to treatment.

KEY WORDS: multiple myeloma, differential diagnostics, X-ray examination

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INTRODUCTION

Today we witness the stunning growth of incidence of tumor diseases, the mortality for which continues to concede only to cardiovascular diseases [3, 4, 5]. The complexity of diagnostics of the disease at initial stages is explained by a fuzzy and unspecific clinical picture, which requires careful differential-diagnostic search [6, 7, 8].

Multiple myeloma (MM) is considered a tumor disease with a low degree of malignancy, which morphological substrate is an uncontrolled synthesis of monoclonal protein, which leads to tumor-like transformations of individual precursor cells [8, 9].

MM is one of the infrequent pathologies, however, the disease is ranked second by prevalence after non-Hodgkin lymphoma (10%) among oncological blood nosologies. American oncologists are afraid that the number of newly diagnosed MM cases may bring the disease to five most common diseases of this profile in the United States. We assume this region of the world not endemic in terms of the oncological pathology, therefore, this trend may loom a global format [11, 12]. In recent years, the fact of underlying genetic anomalies that precondition development and progression of multiple myeloma has been proved, thus explaining family cases [13, 14, 15].

Compared to other oncological diseases, MM is characterized by slow progression, as twenty, and sometimes thirty years [16] may pass from primary tumor-like changes in precursor cells to the onset of clinical symptoms. However, complications associated with acute infectious diseases and renal insufficiency can significantly reduce the life expectancy of patients [17]. The disease is characterized by positive response to treatment and high patient survival rate in the event of early diagnosis and timely treatment [18, 19]. Slow progression and long-lasting latent stage of the disease make the early MM diagnostics a tough case.

In 2014, members of IMWG (International Myeloma Working Group) reviewed and supplemented the diagnostic criteria. The presence of certain criteria undoubtedly confirms the MM diagnosis [1, 2].

MM is characterized by a variety of clinical manifestations. In most cases, the first symptom of multiple myeloma is a subacute continuous pain of migratory nature with a circadian rhythm [20, 21]. Over time, due to metabolic disturbances and formation of myelomic kidney, the patients present with edema syndrome and all other manifestations of chronic renal failure, which has an indolent course in almost 40% of cases [21, 22, 23]. Chronic kidney disease develops as a result of lesion of tubules due to deposition of light chains of immunoglobulins and leads to the development of renal failure. However, onset of the disease with symptoms of chronic kidney disease is quite uncommon [22].

Normochromic anemia develops as one of the hematological manifestations. Symptoms of intoxication [24, 25] come to the fore in the clinical picture of untreated MM cases or in the event of speedy progress of the disease.

Physical changes at the onset of the disease are minimal, with no characteristic features. In the osteolysis areas, bone palpation reveals uneven and painful surface. Hepatosplenomegaly with moderate enlargement of the liver is observed in approximately one third of the cases [26].

THE AIM

The clinical features of multiple myeloma options require a thorough differential diagnostics. The following case history is a vivid example that sustains the thesis. The clinical features of patient K. with MM are presented in this article.

CASE REPORT

Patient K., 58, a resident of the countryside of the Vinnytska region, was admitted to the General Medicine Department of the University Hospital of the Pyrohov Memorial National Medical University (Vinnytsya) on June 04, 2018 with complaints of expressed general weakness, rapid fatigability, shortness of breath and sensation of heaviness in the heart area under physical load, palpitation, edema of the legs, increased body temperature (up to 37.4°C), weight loss by 30 kg over two years, periodic pain in the right hypochondrium, the right hip and knee joints, space-occupying lesion in the left portion of the forehead (associated with a life trauma).

ANAMNESIS MORBI

The patient has noted gradual, unprovoked deterioration of the health status during the last 3-4 months when the above-described complaints emerged. The patient complains of a week-long increased body temperature. According to the patient, she followed the diet and medicine treatment recommended by a traumatologist: the injectable (24 injections) and oral course of a chondroprotectors. She has been continuously taking acetylsalicylic acid (75 mg) and enalaprili maleat (10 mg/day) for the last 6 months. From May 05, 2018 until June 01, 2018, she was admitted to the local district hospital with a diagnosis: IHD; atherosclerotic cardiosclerosis; aortosclerosis; hypertension Grade II; left ventricular hypertrophy; high risk of vascular complications; CF stage IIa, functional capacity II (NYHA); severe anemia of uncertain genesis. The prescribed therapy (torasemid, spironolactone, Corvitin, enalaprili maleat, acetylsalicylic acid, bisoprololi fumarat, and meloxycam) was not sufficiently effective, and the patient K. was discharged home with a slight improvement.

THE MEDICAL HISTORY (ANAMNESIS VITAE)

For a long time, the patient has suffered from chronic cholecystitis. The osteochondrosis of the lumbosacral region of the spine, deforming osteoarthritis of the right hip and both knee joints have been troubling for 5-6 years. She has got a surgery for uterine fibroids (18 years ago) and fracture of the ribs (life trauma 5-6 years ago). For 20 years, she has suffered from chronic kidney disease – pyelonephritis (last exacerbation 2 years ago). The patient denies the presence of viral hepatitis, tuberculosis, HIV, venereal diseases in the medical history; allergic history is not burdened.

OBJECTIVES DATAS

At examination, the state of the patient was of a moderate severity, stable; the patient was conscious, oriented, took active position in the bed. The skin was clean, pale, with a yellowish tinge, warm to the touch, of normal elasticity. Subcutaneous fat was well developed (BMI = 27.2), peripheral lymphatic nodes were not enlarged. Painless tumor-like mass (2 x 3 cm) of tight-elastic consistency was palpable the forehead area. Body T – 36.7°C, RF-20 per minute, BP-150/90 mm Hg, HR-76 bpm.

Nasal breathing was free; clear lung sounds over the lungs against percussion; vesicular breathing without deviation from norm at auscultation. The left cardiac border was shifted 1.5 cm to the left from the middle-clavicular line; the other cardiac borders were not changed; diffuse cardiac impulse. The first cardiac tone at the top was weakened; an accent of the second tone above the aorta was heard. The abdomen of the usual form; superficial and deep palpation was painless. The lower edge of the liver coincided with the edge of the rib arch, rounded, tightly elastic, painless with palpation. The spleen was not palpable. Gall bladder symptoms and peritoneal irritation symptoms were negative. Defecation was normal; the consistency of feces was normal. The Pasternatsky symptom was negative on both sides. Urination – 4-5 times a day, painless. Swelling of the legs of a tightly elastic consistency; no abnormality of color and integrity of the skin of lower extremities was found. The knee joints were slightly deformed. Palpation of the periarticular regions of the right hip and knee joints was moderately painful; the load symptoms were positive; the volume of passive and active movements was somewhat limited; the patient felt a "crunch" when moving.

Given the subjective and objective data, a preliminary diagnosis was established: Severe anemia of unspecified genesis (CKD? Multiple myeloma? Neoplastic process?); CKD Grade 0; chronic pyelonephritis in the remission stage; CKD Grade I; symptomatic arterial hypertension Grade II; hypertrophy of the left ventricle; IHD: diffuse cardiosclerosis, aortosclerosis; CF IIa, II (NYHA); sinus tachycardia; chronic cholecystitis in remission; osteoarthritis; Grade II-III deforming osteoarthritis with a predominant lesion of right hip and knee joints in the stage of unstable remission; Grade 2 arthrosis of metatarsophalangeal articulations (according to the results of the traumatologist's consultation).

Patient K. was examined in accordance with the National Clinical Recommendations for Diagnosis and Treatment of Multiple Myeloma [2, 6]. The examination report was approved by the Local Ethics Committee in accordance with the Declaration of Helsinki (2013).

The patient was assigned additional laboratory and instrumental examinations, the results of which are listed

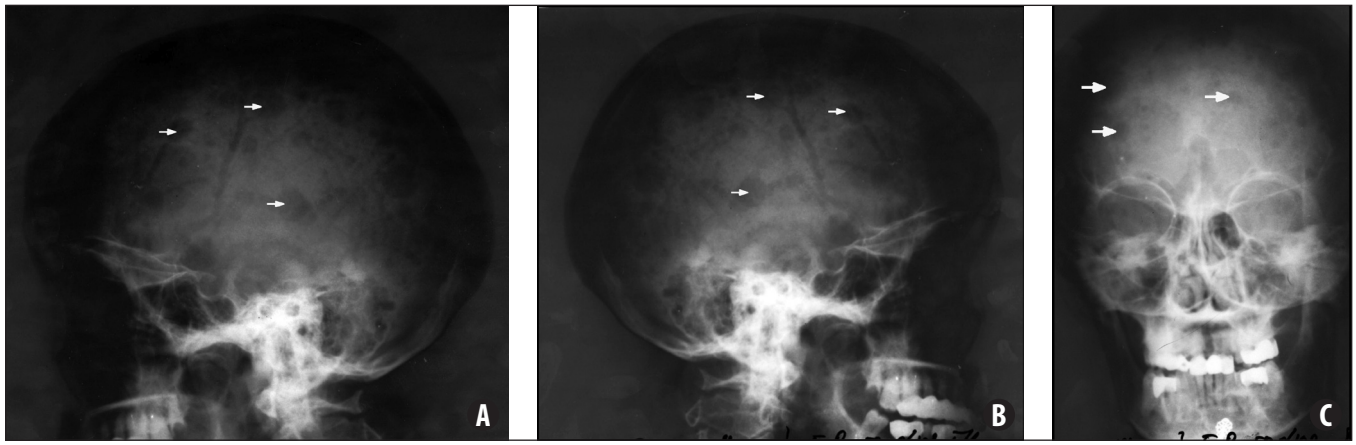

Figure 1. X-ray photos of skull in sagittal left (A), right (B) and front (C) views.
Notes: arrows show multiple focuses of destruction.

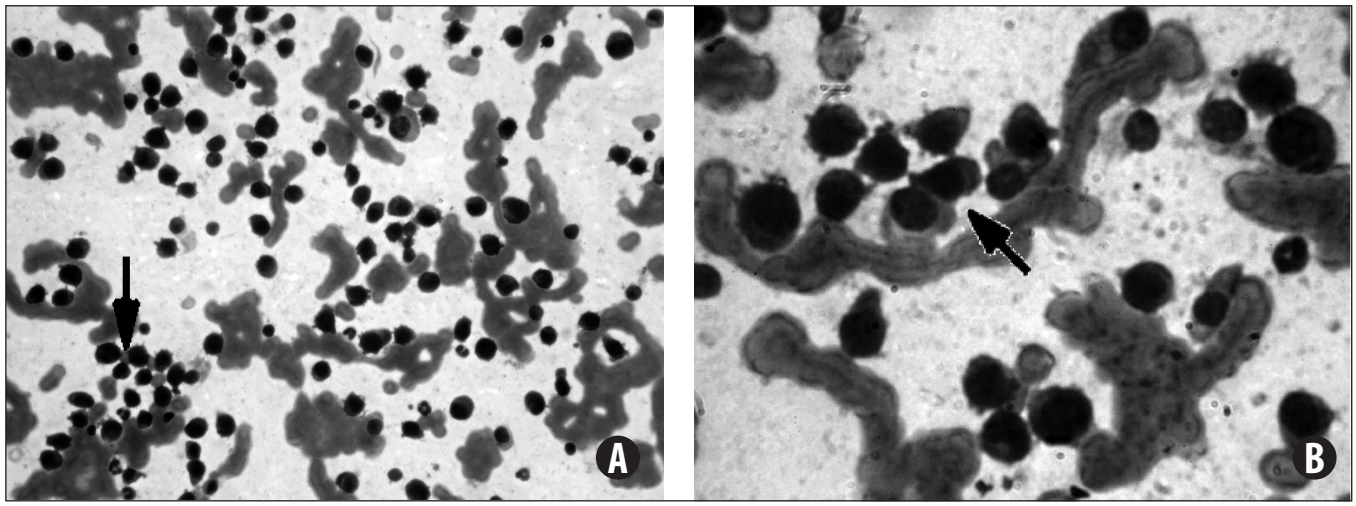

Figure 2. Blood smears photos (A, B).
Notes: arrows show clusters of plasmatic cells.

below. The results of the general blood count suggested the presence of severe hypochromic anemia (Hb – 63 g/l), decreased erythrocyte count ($2.0 \cdot 10^{12}$/l), pronounced anisocytosis, significant ESR growth (82 mm/hour); the leukocyte formula showed a left-handed shift of the formula (plasmatic cells – 2%, young granulocytes – 3%, stab neutrophils – 15%, segmental neutrophils – 55%, lymphocytes – 22%, monocytes – 3%); a slightly decreased platelet count (140×10^9/l). The urinalysis revealed proteinuria ($1.32^0/_{00}$). The biochemical blood parameters, namely the level of bilirubin and its fractions, the intracellular hepatocytic enzymes, the level of total protein, indicators of the functional state of the excretory system and coagulogram were within the norm. The result of Wassermann reaction, viral hepatitis and HIV serological markers were negative. Deviations from the norm were observed as hypercholesterolemia and dyslipoproteinemia (cholesterol – 8.1 mmol/l, B-lipoproteins – 76 IU, triglycerides – 2.0 mmol/l), in the renal excretory function (urea – 10.2 mmol/l, creatinine -120 μmol/l, GFR clearance – 35 ml/min), and indicators of acute phase inflammation (fibrinogen A – 7.6 g/l; fibrinogen B 3+; ethanol test 2+). Significant increase of alkaline phosphatase levels (4882 nmol/l), GGT (2.7 μkat/l), uric acid (880 mmol/l), and hypercalcaemia (3.5 mmol/l) were also observed. The results of the instrumental research were characterized by vast deviations from the normative readings. For example, X-ray examination of the chest revealed signs of age-related changes in the pulmonary tissue, aortic sclerosis, left ventricular hypertrophy, and signs of multiple consolidated fracture of the ribs. The ultrasound examination revealed signs of diffuse changes in the parenchyma of both kidneys and pancreas, fatty involution of the mammary glands, and nodal goiter. An electrocardiographic study recorded left ventricular hypertrophy and sinus tachycardia.

The patient was examined by a surgeon and an ophthalmologist with following opinions presented: fibrolipoma of the frontalis, and retinal angiosclerosis of both eyes, respectively.

The X-ray examination of the skull bones presented isolated multiple destruction phenomena of the round form with a diameter of 2-3 cm with clear contours and signs of osteolysis. The conclusion: signs of multiple myeloma (Fig. 1).

The data obtained narrowed the diagnostic search and the patient was referred for consultation by a hematologist. The sternal puncture revealed 11% of plasmatic cells (Fig. 2).

The diagnosis of multiple myeloma in a patient was confirmed according to the criteria recommended by the International Myeloma Working Group (2017) [6]. The therapy was continued at the hematological department.

DISCUSSION

The classical debut of the multiple myeloma is considered a pain syndrome associated with osteolysis. In elderly patients, one third of all cases of the pathology could be manifested as signs of cardiac and chronic renal insufficiency [4, 21, 22]. However, clinical manifestations of joint pathology, as signs of the bone and muscular system pathology are observed in almost all patients after the age of 60 [27].

In the given clinical case, the complaints of the patient, the history of the disease and life, the results of physical examination and additional research methods, at first glance, corresponded to a picture of chronic cardiac or chronic renal pathologies, or a combination thereof. For example, subjective (weakness, shortness of breath, palpitations, etc.), objective data (displacement of the left cardiac border, the accent of the second tone above the aorta, edema of lower extremities), the results of ECG and X-ray examination of the chest, hypercholesterolemia, and dyslipidemia suggested a cardiovascular pathology. However, slowly progressing edema of the lower extremities, changes in laboratory parameters (severe anemia, high ESR, elevated enzyme count and indicators of acute phase of inflammation and chronic renal failure) were considered as signs of chronic kidney disease. It should be noted that unreasonable subfebrile condition, weight loss, a significant ESR increase, a leukocyte formula left-hand shift, hyperuricaemia and hypercalcaemia did not fit the cardiovascular and excretory system chronic pathology pattern and did not present any answers without additional research. The debut of the disease in the presented clinical case confirmed the data obtained by other researchers regarding MM variants with dominant signs of heart failure and chronic kidney disease [21, 22]. The presence of so-called "red flag" or " alarm" symptoms among the subjective and objective signs in the clinical picture of the patient`s K disease entertained the idea of a possible oncological pathology.

These same ambiguous clinical cases call for an expanded differential diagnostic search with the involvement of specific laboratory and instrumental tests recommended by the WHO clinical protocols.

Special attention of general practitioners regarding MM manifestations should be paid to a combination of the following symptoms and syndromes: moderate and severe anemia, a significant ESR growth, changes in laboratory parameters characteristic for osteolysis and chronic renal failure, even in the absence of characteristic pelvic pain. On the other hand, the resistance of pain syndrome to NSAID therapy also requires a differential diagnostic search. A specific cytological pattern of bone marrow showed by a sternal puncture made it possible to finally confirm the diagnosis of multiple myeloma. The problem of untreated cases of the disease consists in the fact that, on the one hand, patients with unclear clinical picture, the symptoms of osteolysis and the absence of characteristic bone pain rarely agree to sternal puncture, on the other hand, joint pain, changes in laboratory parameters of elderly patients are considered by family doctors as age-related. Therefore, X-ray of flat bones of the skull and pelvis remains one of the simplest available screening diagnostic methods, which allows to detect the osteolysis foci.

CONCLUSIONS

1. Multiple myeloma, especially in recent years, has gained a worldwide trend of growing incidence.
2. Subacute onset, slow progression, various clinical options of the disease, and the presence of comorbid pathology cause difficulties in timely diagnosis of the disease in elderly people.
3. Radiography of flat bones of the skull and pelvis is one of the viable screening methods for detection of multiple myeloma in the primary medical care system.

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