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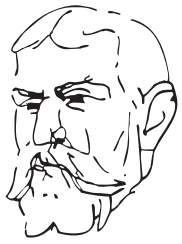

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ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL FEATURES OF THE EXPRESSION OF HUMAN PAPILLOMA VIRUS TYPE 16 IN PLEOMORPHIC ADENOMAS OF SALIVARY GLAND

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ABSTRACT

The aim is to reveal the immunohistochemical features of human papilloma virus type 16 expression in various histological variants of pleomorphic adenomas of the salivary gland.
Materials and methods: The material of the study was surgical and biopsy material from 30 patients with pleomorphic adenomas of the salivary glands, among which in 15 cases mesenchymal was detected, in 10 – mixed, in 5 cases – epithelial histological variant, respectively. Immunohistochemical study was performed, using mouse monoclonal antibody to human papilloma virus type 16. Visualization was performed, using an EnVision™ FLEX detection system. Histological sections of grade III cervical intraepithelial neoplasia (CIN III) were used as a positive control; for a negative control, the procedure was performed without primary antibodies. The immunohistochemical reaction was assessed by a semi-quantitative method by counting the percentage of positively stained cells in the field of view of a microscope × 400. Microspecimens were studied, photoarchived on an Olympus BX-41 microscope.
Results: Expression of human papilloma virus type 16 of varying severity was determined in 26 cases of pleomorphic adenomas of the salivary glands, which was 86.7%. The epithelial component of the pleomorphic adenoma of the salivary gland was characterized by a more pronounced expression of the monoclonal antibody to human papilloma virus type 16 compared to the mesenchymal component of the tumor. The severity of the immunohistochemical reaction with a monoclonal antibody to human papilloma virus type 16 depended on the histological variant of the pleomorphic adenoma of the salivary gland. Epithelial, mixed and mesenchymal variants of pleomorphic adenoma of the salivary gland were characterized, respectively, by the most pronounced, pronounced and moderately pronounced expression of a monoclonal antibody to human papilloma virus type 16.
Conclusions: A comprehensive immunohistochemical study with a monoclonal antibody to human papilloma virus type 16 revealed the presence of a causal relationship between the infection of a patient with human papilloma virus type 16 and development of pleomorphic adenoma of the salivary gland in him.

KEY WORDS: human papilloma virus type 16, immunohistochemistry, pleomorphic adenoma of the salivary gland

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INTRODUCTION

Salivary gland tumors are rare forms of head and neck tumors and benign cases constitute the greatest frequency since only 20 % are malignant. An overall European standardized rate of 4.2-4.9 per 100,000 person-years was reported with a female preponderance (1:1.43) and with an annual 1 % rise in female incidence [1].

Pleomorphic adenoma is the most common salivary gland neoplasm worldwide, accounting for 70-80 % of abnormal growths. It mainly occurs in the superficial lobe of the parotid gland but can also affect the submandibular and minor salivary glands [2].

Etiological factors, causing pleomorphic adenomas, should be well known to minimize their incidence [3]. The importance of human papilloma viruses in the development of pleomorphic adenomas of the salivary glands is a controversial issue. Some scientists note in their studies possible effect of human papilloma virus in pleomorphic adenoma development. On the other hand, some studies do not imply human papilloma virus as a causative agent of salivary gland pleomorphic adenoma [1].

Human papilloma viruses belong to the Papillomaviridae family. Papillomaviruses are one of the most heterogeneous groups of viruses that infect humans and animals. To date, more than 250 papilloma virus types have been identified, and each of these genotypes are associated with infection at particular anatomical sites [4]. Human papilloma viruses, due to differences in DNA sequence, are divided into alpha-, beta-, gamma-, mu- and nu-groups. Human papilloma viruses, taking into account the risk of developing malignant tumors, are classified into viruses of high carcinogenic risk (16, 18, 31, 33-35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70 types) and low carcinogenic risk (6, 11, 42, 43, 44 types) [5].

Oncogenic potential of papilloma virus type 16 is mainly due to the E6 and E7 oncoproteins, as they are key regulators of the cell cycle [6].

Oncoprotein E6 binds protein p53, leading to degradation of the latter by ubiquitin-dependent proteolysis, which results in a violation of the mechanisms controlling proliferation, apoptosis and DNA repair. Interaction of E6 protein with telomerase also enhances the proliferative activity of cells. The E7 oncoprotein action mechanism is associated with functional inactivation of the tumor suppressor pRb, resulting in the release of the transcription factor E2F, which activates genes whose protein products stimulate the cell entry into the S-phase of the cell cycle [7].

THE AIM

The aim is to reveal the immunohistochemical features of human papilloma virus type 16 expression in various histological variants of pleomorphic adenomas of the salivary gland.

MATERIALS AND METHODS

The material of the study was surgical and biopsy material from 30 patients with pleomorphic adenomas of the salivary glands, among which in 15 cases mesenchymal was detected, in 10 – mixed, in 5 cases – epithelial histological variant, respectively. The cut pieces were placed in cassettes. With the help of a cassette holder they were placed in a container for 24-36 hours for fixation in a buffered 10% formalin solution with a pH of 7.4. Conduction and filling the tissue with paraffin was performed using a histoprocessor «HISTOS-5» («Milestone», Italy) on the program for the operating material. After completion of the paraffin impregnation program, the cassettes were removed from the histoprocessor paraffin unit, and at the «HESTION TEC-2800 Embedding Center», the tissue pieces were filled with molten paraffin into molds, followed by solidification on the «HESTION TEC-2800 Cryo Console» refrigeration module. From the obtained paraffin blocks histological sections 3-4 μm thick were made, using a microtome «MICROM HM 325» («Thermo Fisher Scientific», Germany). Immunohistochemical study was performed, using mouse monoclonal antibody (MCA) to human papilloma virus type 16 (clone CAMVIR-1, «Diagnostic BioSystems», USA). Brown staining of cell nuclei characterized positive expression of the marker. Visualization was performed, using an EnVision™ FLEX detection system (Dako, Denmark). Antigen was unmasked in citrate buffer pH 6.0 at 95 °C. Primary antibodies have been incubated at room temperature for 30 minutes, secondary – for 20 minutes. Sections were counterstained with Gill hematoxylin. Histological sections of grade III cervical intraepithelial neoplasia (CIN III) were used as a positive control; for a negative control, the procedure was performed without primary antibodies. The immunohistochemical reaction was assessed by a semi-quantitative method by counting the percentage of positively stained cells in the field of view of a microscope × 400. Microspecimens were studied, photoarchived on an Olympus BX-41 microscope (Japan).

The obtained digital data were statistically processed using the Statistica 10.0 program. The average values were compared, using the nonparametric Mann-Whitney U test. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Among all cases of pleomorphic adenomas studied by us, the immunohistochemical reaction with MCA to human papilloma virus type 16 was positive in 26 cases, i.e. 86.7 %. Analyzing the severity of immunohistochemical expression among 26 cases, it was noted that in 7 – this reaction was mild, in 4 – moderately pronounced, in 15 cases – pronounced. Our data indicate that human papilloma virus type 16 can act as one of the exogenous trigger factors involved in the development of pleomorphic adenoma of the salivary glands.

In our earlier immunohistochemical study with polyclonal rabbit antibody to p16, in 23 cases out of 30 (76.7 %) this marker was found to be expressed in pleomorphic adenomas [8], which is known to be an indirect sign of the human papilloma virus integration of high oncogenic risk into the genome and transformation of epithelial cells into tumor cells [9].

In 26 cases of pleomorphic adenomas, nuclear expression of MCA to human papilloma virus type 16 was determined both in the epithelial (parenchymal) and mesenchymal (stromal) components of the tumor (figure 1). In the tumor parenchyma, a positive immunohistochemical reaction was detected in the nests and cords that formed epithelial cells, as well as in solid, trabecular, cystic, glandular, ductal and tubular structures. A few myoepithelial cells also expressed the above MCA. In the tumor stroma fibroblastic cells, immune cells, vascular endotheliocytes, as well as cellular elements of myxoid and mucoid zones were expressed MCA to human papilloma virus 16.

The positive immunohistochemical reaction found by us in vascular endothelial cells may indicate tropism of human papilloma virus type 16 to the vascular endothelium. Domestic scientists have also revealed a positive reaction in the vessels of the fallopian tubes, which, according to their data, indicates hematogenous spread of the virus in the human body [7].

In the course of a morphometric study, when calculating the percentage of positively stained cells in various pleomorphic adenomas, we found that the maximum, moderate and minimum values, respectively, were in epithelial, mixed and mesenchymal variants of the tumor (table 1). It was also revealed that in epithelial, mixed and mesenchymal variants of pleomorphic adenoma, the epithelial component of the tumor compared with the mesenchymal one was characterized by a large ($p<0.05$) number of positively stained cells.

An equally important issue is the study of human papilloma viruses' effect on the clinical course of pleomorphic adenoma of the salivary glands [9]. In our research, we did not study regularities of the clinical course of salivary glands pleomorphic adenomas, depending on the presence or absence of human papilloma virus type 16.

Table 1. Results of the immunohistochemical reaction in different types of pleomorphic adenoma of the salivary gland

Histological variant of the tumor	Results of the immunohistochemical reaction		
	% positively stained cells	% positively stained cells in epithelial tumor component	% positively stained cells in mesenchymal component of the tumor
Mesenchymal	27.2±3.22 *, ***	45.6±4.02	19.6±2.08 ****
Mixed	56.9±2.87 **, ***	50.5±3.95	21.5±3.19 ****
Epithelial	75.8±4.12 *, **	49.6±2.19	20.9±3.14 ****

* – differences are significant compared to the mixed variant of pleomorphic adenoma,
** – differences are significant compared to the mesenchymal variant of pleomorphic adenoma,
*** – differences are significant compared to the epithelial variant of pleomorphic adenoma,
**** – differences are significant compared to the epithelial component of the tumor.

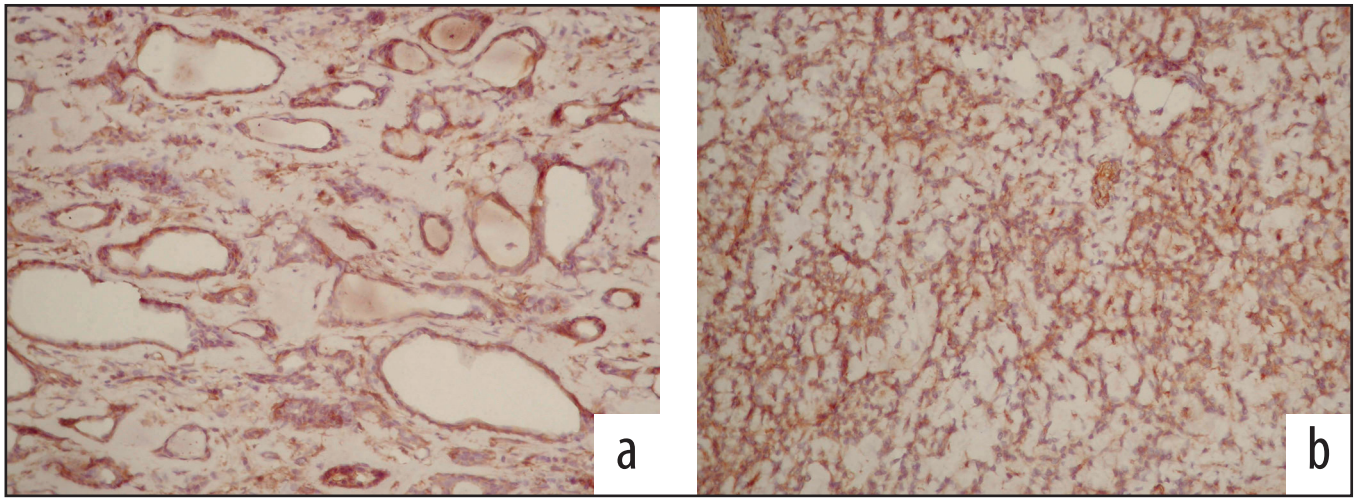

Fig. 1. Expression of MCA to human papilloma virus type 16 in the epithelial and mesenchymal components of the pleomorphic adenoma. Immunohistochemical reaction with MCA to human papilloma virus type 16, a) × 200, b) × 200.

Studies by foreign scientists show that presence or absence of human papilloma viruses in a patient is an independent predictor of survival in malignant tumors of the oropharynx and larynx [5]. It has been noted that human papilloma virus-associated oropharyngeal cancer has a significantly better prognosis compared to other cancers caused by alcohol and tobacco [10].

CONCLUSIONS

Based on our comprehensive immunohistochemical study, the following conclusions can be drawn:

1. Expression of human papilloma virus type 16 of varying severity is determined in 26 cases of pleomorphic adenomas of the salivary glands, which is 86.7%. The obtained data indicate the presence of a causal relationship between the infection of a patient with human papilloma virus type 16 and development of pleomorphic adenoma of the salivary gland in him.
2. The epithelial component of the pleomorphic adenoma of the salivary gland is characterized by a more pronounced expression of the monoclonal antibody to human papilloma virus type 16 compared to the mesenchymal component of the tumor.
3. The severity of the immunohistochemical reaction with a monoclonal antibody to human papilloma virus type 16 depends on the histological variant of the pleomorphic adenoma of the salivary gland. Epithelial, mixed and mesenchymal variants of pleomorphic adenoma of the salivary gland are characterized, respectively, by the most pronounced, pronounced and moderately pronounced expression of a monoclonal antibody to human papilloma virus type 16.

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ORIGINAL ARTICLE

USE OF HERBAL IMMUNOTROPIC MEDICINES FOR COMPLEX TREATMENT OF GENERALIZED PERIODONTITIS IN PATIENTS WITH CONCOMITANT CORONARY ARTERY DISEASE

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ABSTRACT

The aim: Of this study was to improve the efficiency of complex medicamental treatment of generalized periodontitis (GP) in patients with concomitant CAD using of differentiated immunotropic therapy, especially herbal medicine «Immuno-ton».

Material and methods: 130 patients with GP were observed (43 without and 81 with chronic CAD – stable angina, functional classes II-III (CCS)) with detection of oral hygiene indices for Green-Vermillion, inflammation of gums PMA, bleeding of gums PBI, depth of periodontal pockets (determined by direct method). The levels of TNF-α and sPECAM-1 in gingival fluid were detected by ELISA method.

Results: The following article is dedicated to studying on the effectiveness of the proposed method of GP I and II degree of development treatment in patients with a concomitant coronary artery disease (CAD) using of herbal medicines with immunomodulating effect. The offered methods provide disappearance of clinical signs of inflammation in the periodontal tissues and prevention of inflammation recurrence in the long terms. Also, it was proved that usage of the forward method of the GP immunotropic therapy with including of herbal immunomodulators leads to normalization of dynamics of tumor necrosis factor –alfa (TNF-α) and soluble platelet-endothelial cell adhesion molecule -1 (sPECAM-1) in oral fluid of abovementioned contingent of patients.

Conclusions: The progression of generalized periodontitis in patients with stable coronary heart disease is accompanied with manifestation of systemic inflammation, which have been reduced by immunomodulator Immuno-Ton and extratemporal gel with "Enterosgel" and herbal concentrate "Dzherelo".

KEY WORDS: generalized periodontitis, coronary artery disease, immunotropic therapy, cytokines

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INTRODUCTION

Coronary artery disease (CAD) is a major cause of death and disability in developed countries. Although CAD mortality rates worldwide have declined over the past four decades, CAD remains responsible for about one-third or more of all deaths in individuals over age 35 [1, 2, 3, 4].

Periodontitis is a disease of the supporting tissues of the teeth, which are naturally inflammatory [5]. Not only does it affect general health, it also forms a component of the global burden of chronic diseases. Geographical variations are found in the prevalence of periodontal disease and they are mainly attributed to variations in socioeconomic conditions, behavioural factors, systemic conditions of people and oral hygiene patterns [5]. The National Health and Nutrition Examination Survey III (NHANES III) between 1988 and 1994 demonstrated that gingival inflammation occurs in 50% of the adult population [7]. The economic burden of this disease can be estimated from various reports. One such report indicates that periodontal and preventive procedures totalled 14.3 billion dollars, of which, 4.4 billion dollars was spent on periodontal services to directly treat the disease [8].

Issues of the probable link between periodontal disease and CAD have been under active debate for at least the last 20 years. Both ailments are multifactorial inflammatory conditions with many common risk factors (smoking, type 2 diabetes, obesity, etc.), and the key to their progression and development is the comprehensive impact of inflammation [9]. Examination of patients with stable CAD and its unstable variant – acute coronary syndrome, using coronary angiography showed a close relationship with high average indices of periodontal lesions (gingival recession, bleeding after probing, pockets depth etc.), which allowed the authors to connect of both ailments [9].

The explanations for the development and exacerbation of atherosclerotic plaques in periodontitis patients include: (1) bacteremia, (2) a pro-inflammatory state, (3) a prothrombotic state, (4) an overactive immunity, (5) dyslipidemia, and (6) common genetic risk factors. Most likely, these plausible mechanisms play all simultaneously a role [9]. Some immunological factors are involved in the development and control periodontitis, such as: the participation of inflammatory cells in local inflammation, the synthesis of chemotaxis proteins with activation of the complement system and a range of antimicrobial peptides, such as defensins, cathelicidin and saposins. The integration of pathogen-associated molecular patterns (PAMPs) from microorganisms with their surface receptors in the

immune cells, induces the production of several cytokines and chemokines that presents either a pro- and/or anti-inflammatory role by stimulating the secretion of a great variety of antibody subtypes and the activation of mechanisms of controlling the disease, such as the regulatory T cells [6, 10].

Traditional treatment of GP is to eliminate inflammation in the periodontium by removing local irritating factors and use in local and general therapy antimicrobial, anti-inflammatory, antioxidant, osteotropic, antihistamine, vitamin preparations [11]. However, these schemes of treatment rarely supplemented agents that specifically and purposefully influence the immune condition especially in patients with concomitant CAD.

At present, there is widespread interest in the relationship between CAD and periodontitis at the genetic level. One of the first and well-replicated gene loci in coronary heart disease is ANRIL [1]. It is regulatory and does not contain a protein-coding gene. Importantly, it is a pleiotropic gene region that is also responsible for the development of type 2 diabetes, ischemic stroke and Alzheimer's disease [9]. Subsequently established the importance of ANRIL in the development of periodontitis.

Thus, a significant number of interventional, epidemiological and functional studies indicate a possible link between generalized periodontitis and coronary heart disease. Oral bacteria and their products may be involved in all stages of atherogenesis alone or indirectly. Generalized periodontitis, in our opinion, should be regarded as a systemic process that requires not only local but also general treatment.

The following article is dedicated to studying on the effectiveness of the proposed method of GP I and II degree of development treatment in patients with a concomitant CAD using of herbal medicines with immunomodulating effect. The offered methods provide disappearance of clinical signs of inflammation in the periodontal tissues and prevention of inflammation recurrence in the long terms. Also, it was proved that usage of the forward method of the GP immunotropic therapy with including of herbal immunomodulators leads to normalization of dynamics of tumor necrosis factor –alfa (TNF-α) and soluble platelet-endothelial cell adhesion molecule -1 (sPECAM-1) in oral fluid of abovementioned contingent of patients.

THE AIM

To improve the efficiency of complex medicamental treatment of GP in patients with concomitant CAD using of differentiated immunotropic therapy, especially herbal medicine «Immuno-ton».

MATERIALS AND METHODS

130 patients with GP were observed (43 without and 81 with chronic CAD – stable angina, functional classes II-III (CCS)). The diagnosis of periodontitis was verified in accordance of last recommendation [10]. Stable angina was verified due European Cardiology Society (2013) guidelines. All patients signed of Informed consent; design of this trial was approved by local ethic committee.

The exclusion criteria were the presence in patients of cantilever orthopedic structures, dental implants, plate removable dentures, complex dental and jaw abnormalities and deformities; acute coronary syndrome (up to 12 months); stroke, transient ischemic attack (TIA) for up to three months; unstable cardiovascular conditions; implanted intracardiac devices; symptomatic hypertension; alcohol or drug addiction; tumors; known inflammatory diseases of infectious or non-infectious nature; chronic kidney disease (CKD) stage IV-V; liver failure; anemia and other blood diseases; decompensated diabetes mellitus (diabetes); pregnancy and breastfeeding; incapacity and limited capacity; known warnings or contraindications to the use of investigational drugs; refusal to sign an informed consent to participate in the study.

We used the preparation "Immuno-tone" (registration no UA/2179/01/01 from 03.11.2009) and phytoconcentrate "Dzherelo" (registration no. 02568182/031880/01 dated 05.02.2009). Immuno-tone contains eleutherococcus extract, tincture of rhizomes with purple echinacea roots, tincture of hypericum; this drug has a combined adaptogenic and immunomodulatory action due to biologically active substances of medicinal plants. "Immuno-tone" increases the body's natural forces against the influence of adverse environmental factors, stimulates the immune system, gently tones the central nervous system. The immunomodulatory effect is due to the stimulation of mainly cellular immunity, an increase in the number of T-lymphocytes, an increase in the phagocytic activity of leukocytes, the release of some cytokines and is used in chronic recurrent inflammatory diseases. Phytoconcentrate "Dzherelo" was created on the basis of composition of food and medicinal plants. The components of the drug activate the processes of energy formation in cells, stimulate redox reactions, promote the regeneration of cellular organelles, as well as stimulate local nonspecific immunity of the oral cavity.

The therapeutic efficacy of the drugs was determined by their influence on the subjective and objective clinical features of the GP course (oral hygiene indices for Green-Vermillion, inflammation of gums PMA, bleeding of gums PBI, depth of periodontal pockets (determined by direct method).

The levels of TNF-α and sPECAM-1 in gingival fluid were detected by ELISA method with use of TNF-α ELISA kit (Vector-Best, Russia) and sPECAM-1 ELISA BMS229 kit (Bender MedSystems, Austria). Statistical analysis was performed with Statistica system software, version 12.0. Categorical variables are presented as percentages, whereas continuous variables are presented as mean (M) and standart error of mean (m) if normally distributed, or as median and interquartile range (Me [IQR]), if not. Categorical variables were compared by the $\chi 2$ test and continuous variables by the t test or the Mann–Whitney U test. A p value of <0.05 was considered statistically significant. All tests were 2-sided.

All observed patients were divided into following groups due to stage of GP development, presence of concomitant CAD and algorithm of proposed treatment: 1A (10 persons) – patients with generalized periodontitis I stage which received standard treatment; 1C (22 persons) – patients with generalized periodontitis I stage and stable angina FC II-III which received standard treatment of both diseases and additional instillation to periodontal pockets of extratemporal gel with "Enterosgel" and herbal concentrate "Dzerelo"; 2A (10 persons) – patients with generalized periodontitis II stage which received standard treatment; 2C (22 persons) – patients with generalized periodontitis II stage and stable angina FC II-III which received standard treatment of both diseases and additional instillation to periodontal pockets of extratemporal gel with "Enterosgel" and herbal concentrate "Dzerelo"; 1B (10 persons) – patients with generalized periodontitis I stage which received standard treatment and immunomodulator "Imuno-ton" (GalychPharm, Ukraine) 15 ml, twice a day, in the morning, after meals; 1D (23 persons) – patients with generalized periodontitis I stage and stable angina FC II-III which received standard treatment of both diseases and additional instillation to periodontal pockets of extratemporal gel with "Enterosgel" and herbal concentrate "Dzherelo", and immunomodulator "Imuno-ton" 15 ml, twice a day, in the morning, after meals; 2B (10 persons) – patients with generalized periodontitis II stage which received standard treatment and immunomodulator "Imuno-ton" 15 ml, twice a day, in the morning, after meals; 2D (23 persons) – patients with generalized periodontitis II stage and stable angina FC II-III which received standard treatment of both diseases and additional instillation to periodontal pockets of extratemporal gel with "Enterosgel" and herbal concentrate "Dzerelo", and immunomodulator "Imuno-ton" 15 ml, twice a day, in the morning, after meals.

Evaluation of treatment efficacy was performed immediately after the proposed treatment and at 3, 6 and 12 months of follow-up.

RESULTS

Patients with GP I and II stages of development and concomitant CAD after 2 – 3 sessions had an improvement in the condition of periodontal tissue in relation to patients' complaints: halitosis, decreased feeling of discomfort or gum pain, normalization of gum color, disappeared or changed of gums bleeding, changing nature or disappearing exudate. Objective examination showed a dynamic decrease in the symptoms of symptomatic gingivitis, in most patients before 3 sessions completely stopped exudation from the periodontal pockets The mucous membrane of the gums became dense, became pale pink in color, decreased recession, swelling and bleeding on palpation. The timely conducted of curettage (with the second stage of GP development) in the complex treatment led to a decrease in the depth of the periodontal pockets, termination of exudation and epithelialization of the bottom. Normalization of clinical parameters in all groups of patients, regardless of the proposed treatment, occurred at 3-4 visits. The number of treatment sessions depended on the degree of GP development and the severity of symptomatic gingivitis. The average number of sessions in subgroups of patients with GP I and II stages of development using the proposed method of treatment was 3.25 ± 0.42; in subgroups of patients with GP I and II stages of development using traditional treatment – 3.75 ± 0.47. At the end of treatment, objective examination showed normalization of the color, texture and configuration of the gums.

It should be emphasized that the results obtained demonstrate a qualitative and equal randomization of patients by groups.

One of the main criteria for the severity of the inflammatory process in the periodontium is the manifestation of symptomatic gingivitis, which can be evaluated by the dynamics of the PMA inflammation index and bleeding PBI.

During the main (1B, 1D) and comparison subgroups (1A, 1C), positive PMA dynamics were observed during treatment, the area of tissues covered by inflammation decreased from (31.48±0.75)% and (34.64±0.86)% to (4.6±0.4)% and (4.5±0.4)% – in subgroups 1B and 1D respectively; from (32.65±0.77)% and (34.15±0.85)% to (5.3±0.5)% and (5.1±0.5)% in the 1A and 1C respectively. However, in subgroups 1B and 1D, this process proceeded more efficiently, remaining at 9.84; 11.24; 5.52 times and 9.62; 10.83; 6.66 times lower than the baseline by 3, 6 and 12 months respectively, whereas in the comparison subgroups 1A and 1C, this indicator decreased in the corresponding time in 5.27; 4.8; 1.94 times and 5.5; 5.17; 2.07 times with statistically significant difference between subgroups at 3, 6 and 12 months ($p < 0.001$). Immediately after treatment, there was a 3-times decrease in the PBI index in the main subgroups and 2.5-times in the comparison subgroups, which proves the clinical effectiveness of the treatment in each subgroup. Significant difference of indicators was found with respect to the index of bleeding of PBI for 3 months in the main subgroups 1B and 1D – a decrease in 3.8 times ($p<0.001$) compared to the initial values; in comparison subgroups 1A and 1C, this indicator decreased in 2.4 and 2.59 times respectively ($p < 0.05$). At 3, 6 and 12 months, a clear significant difference in the magnitude of the PBI bleeding index was established between groups ($p < 0.001$).

Indicators of bleeding indices and PMA are directly correlated with indicators of oral hygiene and its changes in the course of treatment and preventive measures.

At the stage immediately after the treatment, a positive dynamics was observed in terms of periodontal pockets depth, also. Periodontal pockets decreased within 0.76 mm and 1.12 mm in the major subgroups, 0.21 mm and 0.64 mm in the comparison subgroups. This indicator remained stable in dynamics in the main subgroups during other periods, so for 3, 6 and 12 months the periodontal pockets depth in subgroup 1B decreased to 0.78 mm, 0.66 mm and 0.64 mm respectively; in subgroup 1D – decreased to 1.19 mm, 0.93 mm, and 0.9 mm respectively, significantly different from similar values in the comparison subgroups ($p < 0.001$), indicating remission of the dystrophic-inflam-

matory process in the periodontium.

In the study of index indicators between the main and the comparison group, the dynamics of changes that characterize the activity of the pathological process in periodontal tissues in patients with GP II stage of development and with concomitant coronary heart disease at the stages of treatment were detected, compared with the initial data and between groups. As a result of the clinical examination and index evaluation, it was found that in the main and in the comparison group, directly after the complex treatment in comparison with the initial data, there is a significant decrease in a number of indicators (periodontal pockets depth, PBI index, PMA, hygienic condition, tooth mobility). In general, analyzing the data obtained, it can be stated that the treatment regimens for patients with GP in both groups are adequately selected, as evidenced by some clinical effect.

Positive dynamics of PMA index was observed in the main and in the comparison group: in subgroups 2B and 2D respectively (45.8±0.93)% and (44.4±0.91)% to (5.48±0.4)% and (5.6±0.4)%; in subgroups 2A and 2C to (45.5±0.93)% and (45±0.93)% to (6.51±0.53)% and (6.75±0.5)%. However, in the main subgroups this process was more efficient and maintained positive dynamics, remaining below baseline in subgroup 2B for 3 months – 9.35 times, 5.39 and 3.39 times for 6 and 12 months respectively; in the 2D subgroup 3 months – 8.88 times, 4.53 and 3.22 times 6 and 12 months respectively. Whereas in the comparison group this indicator decreased in the corresponding terms – in the subgroup 2A for 3 months – 6.23 times, 4.0 and 2.37 times for 6 and 12 months respectively; in the 2C subgroup at 3 months – 6.34 times, 3.3 and 2.4 times at 6 and 12 months respectively ($p<0.001$).

There was a decrease in the bleeding index of PBI (in the main group – 4.8, in the comparison group – 3 times), which proves the clinical effectiveness of the conducted treatment in each group, as well. A clear difference in the PBI indexes was found at 3 months – a decrease in 10.38 times in subgroup 2B and 8.84 times in subgroup 2D ($p <0.001$), whereas in subgroups 2A and 2C this indicator decreased in 3.93 and 3.86 times respectively ($p <0.001$). At 3 months there was a significant difference in the value of bleeding index between subgroups ($p <0.05$), at 6 and 12 months ($p <0.001$).

6 months after the treatment was observed stabilization of periodontal tissues condition in all subgroups, with exception of isolated cases.

The level of TNF-α in gingival fluid of patients with stable angina was higher for 22.13% and for 32.29% in observed patients with generalized periodontitis I and II stage relatively ($p<0.05$). Its concentration was significant decreased after treatment in all groups, but more strong in patients with additional use of immunomodulators (see table 1). In patients with angina pectoris normalization of TNF-α levels was more slowly compared group without coronary atherosclerosis.

Similar results were obtained accordance of levels of sPECAM-1 in gingival fluids of observed patients (see table 2).

DISCUSSION

Using of immunomodulating medicines of herbal origin contributes of local and systemic immunity normalization, inhibition of interdental and interradicular septas inflammatory resorption and stimulation of periodontal tissues reconstruction speed [6, 7].

It is reasonable to prefer medicines with many-sided activity, that afford to have an effect on different aspects of aetiology and pathogenesis of disease for optimization of complex therapy efficiency, taking into account polyaetiology of GP [5].

The resulted data confirming that GP with concomitant CAD develops on a ground of local and systemic immunity disturbances. Prominent changes of proinflammatory cytokines in oral liquid of examined contingent of patients may be explained by both side influence (GP – CAD) on compensatory mechanisms, which regulate homeostasis of immune system. [9].

It's known, TNF-α is a pleiotropic pro-inflammatory cytokine released by a variety of different cell types in response to various stimuli, including bacteria, parasites, viruses, cytokines and mitogens. It is involved in systemic and local inflammation via different signal pathways, inducing a broad range of genes. TNF-α regulates a host response to infection and its deregulation is implicated in the pathogenesis of numerous complex diseases, including periodontitis. This cytokine was shown to drive several biological processes such as induction of inflammatory mediators, for instance, matrix metalloproteases, chemokines and prostaglandins, endothelial cell activation and endothelial-leukocyte interactions, monocyte adhesion, mediating bone remodelling, and oxidative processes [12, 13]. TNF-α induce bone resorption and up-regulate prostaglandin E2 (PGE2) and collagenases secretion and is produced by many cell types including macrophages, neutrophils, keratinocytes, fibroblasts, NK cells, T and B cells in the periodontium. Thise cytokine induces the up-regulation of adhesion molecules on leucocytes and endothelial cells, stimulating the production of chemokines that recruit circulating leucocytes to sites of inflammation, and inducing expression of other inflammatory mediators that potentiate inflammatory responses [13]. PECAM-1/CD31is a 130-kD vascular cell adhesion and signaling molecule of the immunoglobulin (Ig) superfamily that is expressed on the surface of circulating platelets, monocytes, neutrophils, and selected T-cell subsets. It is also a major constituent of the endothelial cell intercellular junction and plays a role in neutrophil recruitment at inflammatory sites. There is good evidence to suggest that PECAM-1 is a key participant in the adhesion cascade leading to extravasation of leukocytes during the inflammatory process [14]. Many studies suggest that chronic periodontitis is an independent risk factor for systemic vascular disease and may result in stimulation of the synthesis of acute phase protein by cytokines released by periodontal high endothelial cells [15]. However, tissue expression of adhesion molecules has not been substantially evaluated in the gingiva of patients with chronic periodontitis. This is significant in relation to potential therapy targeting expression of the adhesion molecules [14].

Table I. Dynamics of gingival fluid levels of TNF-α (pg/ml) in patients with generalized periodontitis

Period	Groups of observed patients							
	1A, n=10	1C, n=22	2A, n=10	2C, n=22	1B, n=10	1D, n=23	2B, n=10	2D, n=23
Before treatment	41.56±2.45	54.63±3.41	51.24±2.97	67.44±2.31	44.33±2.67	55.67±3.47	52.47±2.95	69.76±2.54
1 month	32.99±3.52 P<0.01	41.14±2.91 P<0.01	43.14±3.23 P<0.01	53.67±2.77 P<0.01	27.35±2.21 P<0.01	37.57±2.89 P<0.01	39.77±3.12 P<0.01	51.47±3.32 P<0.01
3 month	31.47±2.78 P<0.01 P^1>0.05	39.97±3.11 P<0.01 P^1>0.05	41.11±2.54 P<0.01 P^1>0.05	51.23±3.14 P<0.01 P^1>0.05	25.67±2.78 P<0.01 P^1>0.05	36.77±2.91 P<0.01 P^1>0.05	35.67±3.11 P<0.01 P^1>0.05	46.77±3.98 P<0.01 P^1>0.05
6 month	29.46±3.71 P<0.01 P^1>0.05 P^2>0.05	36.82±3.23 P<0.01 P^1>0.05 P^2>0.05	37.79±3.45 P<0.01 P^1>0.05 P^2>0.05	49.67±2.23 P<0.01 P^1>0.05 P^2>0.05	26.14±3.22 P<0.01 P^1>0.05 P^2>0.05	31.54±3.43 P<0.01 P^1<0.05 P^2<0.05	32.13±3.34 P<0.01 P^1<0.05 P^2>0.05	47.14±3.37 P<0.01 P^1>0.05 P^2>0.05
12 month	30.47±2.87 P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	38.91±2.11 P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	38.11±3.31 P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	48.45±3.11 P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	26.74±2.31 P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	30.89±2.78 P<0.01 P^1<0.05 P^2<0.05 P^3>0.05	32.76±2.98 P<0.01 P^1<0.05 P^2>0.05 P^3>0.05	43.56±2.99 P<0.01 P^1<0.05 P^2<0.05 P^3<0.05

Notes: p – significance in compared groups during follow-up period

Table II. Dynamics of gingival fluid levels of sPECAM-1 (ng/ml) in patients with generalized periodontitis

Period	Groups of observed patients							
	1A, n=10	1C, n=22	2A, n=10	2C, n=22	1B, n=10	1D, n=23	2B, n=10	2D, n=23
Before treatment	104.45 [91.34; 119.54]	128.77 [111.43; 136.74]	107.87 [93.45; 121.42]	147.45 [121.23; 167.42]	107.11 [94.75; 129.25]	135.78 [121.47; 147.98]	114.45 [91.42; 135.87]	146.21 [120.12; 165.32]
1 month	75.78 [55.34; 99.67] P<0.01	97.56 [87.45; 117.32] P<0.01	91.21 [75.45; 108.23] P<0.01	107.67 [89.78; 114.11] P<0.01	61.45 [48.78; 87.98] P<0.01	91.78 [79.54; 103.22] P<0.01	90.42 [74.74; 106.23] P<0.01	91.45 [78.79; 102.12] P<0.01
3 month	74.23 [56.34; 96.45] P<0.01 P^1>0.05	93.52 [83.41; 112.34] P<0.01 P^1>0.05	89.78 [76.45; 107.54] P<0.01 P^1>0.05	106.35 [91.25; 112.74] P<0.01 P^1>0.05	54.45 [38.44; 74.11] P<0.01 P^1<0.05	81.78 [65.47; 92.23] P<0.01 P^1<0.05	79.79 [64.51; 91.88] P<0.01 P^1<0.05	76.51 [54.89; 98.14] P<0.01 P^1<0.05
6 month	71.23 [55.76; 98.34] P<0.01 P^1>0.05 P^2>0.05	92.44 [85.42; 109.85] P<0.01 P^1>0.05 P^2>0.05	87.34 [72.45; 105.15] P<0.01 P^1>0.05 P^2>0.05	104.12 [92.12; 11.85] P<0.01 P^1>0.05 P^2>0.05	53.74 [39.78; 73.45] P<0.01 P^1<0.05 P^2>0.05	79.89 [66.45; 91.74] P<0.01 P^1<0.05 P^2>0.05	78.14 [63.61; 90.45] P<0.01 P^1<0.05 P^2>0.05	75.16 [56.14; 98.27] P<0.01 P^1<0.05 P^2>0.05
12 month	69.34 [49.34; 80.34] P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	83.78 [63.45; 105.15] P<0.01 P^1<0.05 P^2<0.05 P^3<0.05	88.78 [73.87; 104.75] P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	105.57 [93.45; 113.75] P<0.01 P^1>0.05 P^2>0.05 P^3>0.05	54.87 [38.78; 73.45] P<0.01 P^1<0.05 P^2>0.05 P^3>0.05	80.42 [67.45; 94.77] P<0.01 P^1<0.05 P^2>0.05 P^3>0.05	80.11 [66.63; 93.14] P<0.01 P^1<0.05 P^2>0.05 P^3>0.05	74.58 [53.71; 95.98] P<0.01 P^1<0.05 P^2>0.05 P^3>0.05

Notes: p – significance in compared groups during follow-up period

CONCLUSIONS

1. The progression of generalized periodontitis in patients with stable coronary heart disease is accompanied with manifestation of systemic inflammation, which is evidenced by the increase in oral fluid levels of TNF-α (in 2.28 and 2.86 times for I and II stages) and sPECAM-1 (in 2.66 and 2.99 times respectively). Strong positive relationship between TNF-α and sPECAM-1 content in oral liquid and PMA inflammation indices was detected.
2. The use of herbal immunotropic drugs in the complex treatment of generalized periodontitis in patients with stable coronary heart disease reduces its clinical manifes-

tations (PMA – on 79.4%, PBI – on 63.2%, depth of periodontal pockets – on 27.4%), improves the immunogram, reduces the manifestations of systemic inflammation (the level of TNF-α in the oral liquid decreased by an average on 27.3%). It was found that in the long term (after 6 and 12 months) after treatment, the stabilization of periodontal tissues conditions persists and there is a decrease in the number of relapses, by an average on 24.2%.

3. Additional prescription of immunomodulator Immuno-Ton and extratemporal gel with "Enterosgel" and herbal concentrate "Dzherelo" caused more significant normalizing of local inflammation activity in patients with generalized periodontitis and concomitant CAD.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

MEDICAL STAFF IN THE CARE OF A PREGNANT PATIENT DIAGNOSED WITH FETAL DEFECT

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ABSTRACT

The aim: The research conducted aims at evaluating the way the medical staff functions in the care of the pregnant patient diagnosed with fetal defects.

Material and methods: 158 midwives took part in the study. The MINI-COPE standardized questionnaire and author's questions were used.

Results: During the period when the care was provided for the pregnant patients diagnosed with fetal defects, nearly 50% of the midwives under examination complained about the decline of their wellbeing. 75,3% of the respondents seek help and advice on how to deal with a stressful situation. Ideal for providing a systemic support, the midwives most often chose a psychologist and a psychotherapist. The surveyed who reported no need for a systemic support for the medical staff at the workplace, as the causes of their attitude pointed to their diminished trust in the ward nurse and no place for honest conversations. Most of the midwives surveyed during the care of the patient with the fetal defects experienced stress and bigger mental exhaustion. Over half of the surveyed reported lack of systemic psychological support provided for the medical staff at the workplace.

Conclusions: The research indicates the need to introduce courses and training on topics related to how to deal with stressful situations into the education system of medical staff.

KEY WORDS: medical staff, prenatal care, obstetric nursing, fetal diseases, psychological stress

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INTRODUCTION

The progress of medical science, including obstetrics, has set new tasks for medical personnel. This involves raising qualifications, gaining new powers and increasing independence of therapeutic team members. The incidence of abnormal course of pregnancies is increasing, which increases the challenges for a midwife, an obstetrician, but also the patient with diagnosed fetal defect. These pregnancies are subject to more frequent monitoring and are associated with the necessity of hospitalization at an earlier stage, caused by a child's state of health, a mother's or both. During a patient's pregnancy, an important role of a midwife is to ensure mutual communication and provide holistic care, which she initiates with her cooperation with a doctor, psychologist and family. A midwife should be characterized by sensitivity to the patient's problems, as well as efficiency in taking preventive actions and managing health problems [1].

The World Health Organization (WHO) defines congenital malformations as structural or functional abnormalities that occur in utero (intrauterine) and that are detected either in the prenatal period, at birth, or later, in early childhood [2]. The occurrence of birth defects in live-born children is estimated at 2-3%. This number increases to 10% among stillborn births and up to 70% of miscarriages occurring in the early stages of pregnancy.

Genetic and environmental factors are recognized as the causes of birth defects. Differentiating birth defects in the fetus, we divide them into large (also lethal), with serious consequences for the health of the fetus, usually ending with medical interventions, and small ones. With the abovementioned defects, the number of deaths increases to infancy, as well as the need for hospitalization.

Thanks to the progress of medicine, development in obstetric and prenatal care, we have the possibility of early recognition of any disorders and defects. Changing the procedure for a patient with a diagnosed fetal defect is an immeasurable benefit. The standard of care also requires multidisciplinary cooperation in obstetric, nursing and medical teams. As a result, also cooperation with parents may change [3]. A number of decisions regarding the subsequent management of the child after delivery may involve extreme emotions in ethical and moral feelings. Unfavorable diagnosis disturbs a number of plans related to motherhood.

This situation poses challenges also for the medical staff [4, 5]. The literature extensively describes and analyzes issues related to stress, burnout and excessive employee involvement. Facing health care personnel with the phenomenon of death causes a number of consequences, which is, among others deep sadness or a sense of loss. These reactions may be associated with helplessness and a greater need to show concern [6, 7]. The specificity of work and tasks in the medical sector resulting from legal, organizational and ergonomic concepts affect workload. Working conditions affecting these concepts, such as time pressure, physical and mental effort (participation in human tragedies), employer support and the financial

factor, show the danger of stress, post-traumatic stress and burnout among medical personnel [8-10].

Currently, the midwife's professional independence is gaining a new dimension. The particular risk of stress influences the psychophysical fitness of midwives; the consequence of which is health exposure, lower satisfaction and motivation from work [11-13]. The possibility of vocational education raising the qualifications of midwives, as well as greater professional awareness, does not blur the customs and traditions in the specificity of work performed and does not comply with applicable care standards [8,11]. The remuneration system does not compensate for work in the profession of a midwife in which contact with the patient is dynamic, characterized by specific principles of creating a sense of trust [11]. The implementation of professional tasks and lack of support from superiors, the therapeutic team they work with, employers and the society increase the degree of workload [10,14-16]

In the literature, "coping with stress" appeared in the sixties [17]. Stress affects the mental regulation system, which is why the process of coping with stress is important. It is necessary to maintain human behavioral and mental balance [17,18].

The midwife's work is particularly burdened for reasons of participating in surgical deliveries, including termination of pregnancy, work with people with emotional load, or because of moral doubts. It can result in a lack of job satisfaction, or even stress conducive to burnout [19]. Increasingly, you can come across the problem of court cases, where the accused are midwives and / or obstetrician [20].

That is why the support we can get from the self-help group turns out to be so important. Medical workers get the greatest support related to stress at work in the bio-psychic renewal group, where awareness and self-knowledge are expanded. A consultant in such a group is a clinical psychologist [17].

THE AIM

The main objective of the study was to assess the functioning of midwives in caring for a patient with a diagnosed fetal defect.

Research issues:

1. Analysis of systematic and related to psychological experiences support received by midwives in the care of a patient in whom a fetal defect was found.
2. Assessment of the influence of midwives' sociodemographic factors in caring for a patient with a fetal defect.
3. Assessment of stress management strategies in midwives in the care of a patient with a fetal defect.

MATERIAL AND METHODS

The research was conducted in the period from October 2018 until March 2019; 158 women took part in it. The selection for the group was random. The respondents were informed that the survey is completely voluntary and anonymous, and the results obtained will be used for scientific purposes. The material was collected by means of a diagnostic survey, survey technique, using the standardized MINI-COPE questionnaire. The survey consisted of 40 questions. The first part contained the sociodemographic characteristics of the respondents, the second part was the Mini-COPE questionnaire, while the third part of the survey consisted of author's questions.

The COPE Inventory is used for measurement of Coping with Stress. It contains 28 statements included in 14 strategies (2 statements in each strategy), which are: active coping, planning, positive re-evaluation, acceptance, sense of humor, turning to religion, seeking emotional support, seeking instrumental support, doing something different, denying, discharging, taking psychoactive substances, stopping actions, blaming oneself.

RESULTS

The significance of differences was determined by carrying out the Kruskal-Wallis test for independent groups. The Pearson chi square test was used to assess dependencies in surveys. Statistical analysis was performed using Statistica v 10.0 Statsoft, assuming a 5% probability of error.

The sociodemographic analysis concerned age, education and seniority of the respondents. The majority of midwives surveyed – 44.3% were in the age group up to 30 years of age, another 41.8% were midwives in the 31-55 age range, while the least 13.9% of the surveyed were over 51 years old. Among the respondents, 39.9% of midwives had a master's degree or specialist, 33.5% completed medical study of midwifery or bachelor's degree, while 26.6% of midwives holding a bachelor's or master's degree had an additional specialization. The largest group were midwives with up to 5 years of seniority – 40.5%, while the smallest with 11-20 years of experience – 15.8%. The vast majority of midwives surveyed – 65.2% worked in hospitals with the 3rd degree of reference.

The analysis of the responses to the questions contained in the author's questionnaire is presented in Table 1.

Analyzing the relationship between work experience and the experience of stress during the care of a patient with a fetal defect, a statistically significant difference was found ($p = 0.014440$). Most often, stress in such a situation is experienced by midwives working for 5 years (91% of this group). There is also a statistically significant relationship ($p = 0.005834$) between work experience and participation in courses or training aimed at coping with difficult situations in the ward; midwives working for over 20 years most often participated in such courses. The analysis showed that there is also a statistically significant relationship ($p = 0.001081$) between work experience and the knowledge of the functioning of systemic psychological support for medical personnel. The most aware of the lack of such support are midwives working for up to 5 years; women with 11-20 years of work experience most often do not know if such support exists in their workplace.

Table 1. Responses given by the respondents in the author's questionnaire.

Questions contained in the questionnaire	Responses given by the respondents (in percent)		
	Yes	No	I do not know
When looking after a patient with a diagnosed fetal defect, do you experience greater mental fatigue than usual?	80	15	5
When looking after a patient with a diagnosed fetal defect, do you experience deterioration of physical well-being?	47	39	14
Do you feel stress while taking care of a patient with a diagnosed fetal defect?	81	15	4
Did you participate in courses / trainings that were aimed at dealing with difficult situations in the ward?	32	68	-
Does your workplace have systemic psychological support for medical staff?	18	64	18
Should there be systemic support for medical personnel?	95	1	4

Source: own study.

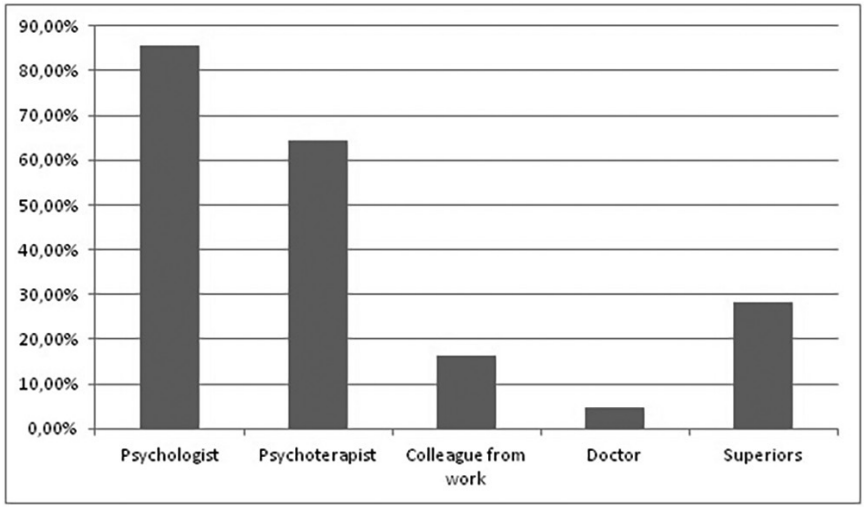

Fig. 1. A list of people ideal for providing systemic support to medical personnel.

Source: own study.

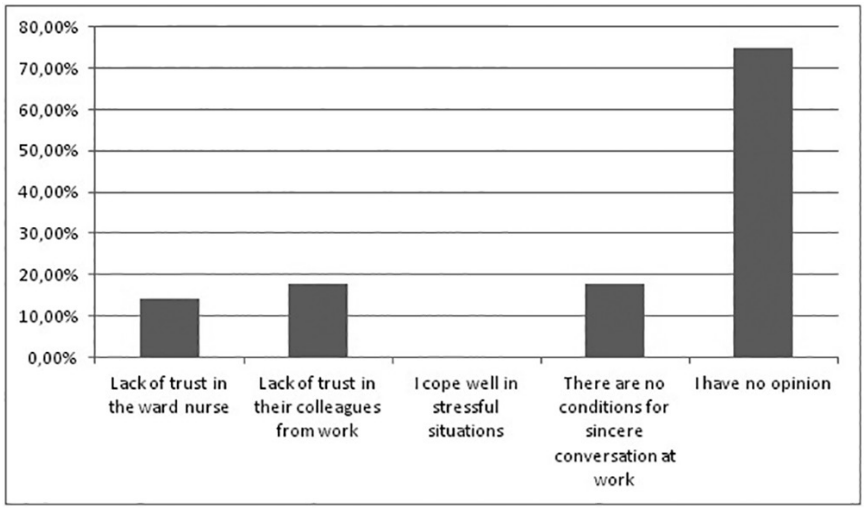

Fig. 2. A list of reasons for the lack of willingness among midwife respondents for the functioning of systemic support for medical staff in the workplace.

Source: own study.

In response to the question about the desire to receive systemic support in the workplace, the midwives indicated persons who would ideally provide such support. The most frequently mentioned mentors – 85.5%, psychotherapist – 64.5%, supervisor – 28.3%. In turn, 16.4% of the respondents considered their colleague from work and 4.6% – the doctor, as persons suitable for providing support (Fig. 1).

17.9% of midwives, as a reason for the lack of willingness to have systemic support for medical personnel operated in the workplace, stated lack of trust in their colleagues and recognized that there are no conditions for honest conversation at work. 14.3% of respondents as the reason stated lack of trust in the ward nurse (Fig.2).

The most important information obtained thanks to the analysis of the results of the MINI-COPE questionnaire is presented below:

1. A positive phenomenon was observed, which was avoidance of stimulants. The vast majority of midwives surveyed – 72.2% showed that they almost never took alcohol or other drugs to feel better in a stressful situation. It is satisfactory that none of the respondents indicated the answer "almost always" and that only 6.3% of respondents often chose these stimulants.
2. In the case of achieving the intended goal, the examined midwives rarely – 46.2% or almost never – 39.9% gave up attempts to achieve it.
3. The vast majority of the surveyed women – 75.3% seek advice and help from others regarding to what should be done, 19.6% of midwives almost always developed such a strategy or plan of action for coping with stress. Only 4.4% of respondents did so rarely or almost never – 0.6%.
4. Another way to deal with stress, was to seek solace in their faith. The responses of the midwives surveyed were very diverse: 34.8% of women almost never chose such a way, rarely – 29.1%, often – 24.1% and nearly always – 12.0%.

DISCUSSION

Based on our own results, it was found that while caring for a patient with a diagnosed fetal defect, almost half of the midwives surveyed declared a deterioration in physical well-being. Nowakowska et al. [21] in her research presents similar results. 39% of nurses participating in the survey complain of physical exhaustion during work. The analysis of own research showed that 63.9% of midwives do not have systemic support for medical workers in the workplace. This was also confirmed by the research of Saiful-Islam et al. [22] conducted in nursing homes in Great Britain. The presented research showed that employees of primary health care in difficult situations could not count on the support of superiors. The vast majority of respondents, as much as 79.9%, declared greater mental fatigue. Similar results were obtained by Nowakowska et al. [21], in which 65% of nurses declared their work as emotionally exhausting. In the Mróz [23] study, emotional tension is consistent with the overloaded type, and negative correlation in functioning with unambitious type.

The author compares her research with the results of Golińska, in which different correlations were observed. In the theory of work-related behaviors and experiences, Maslach notes the relationship between feeling of stress and burnout, in which she details emotional exhaustion [23].

At the same time, our own research has noted that there is a very high demand for systemic support for medical personnel. As much as 96% of respondents indicate the essence of implementing such support. Similar observations were made by Banasiewicz [19], where 56% of midwives report lack of professional support.

Comparable results were obtained by Kluczek et al. [24], in which ¾ respondents also noticed the need to introduce employee training in dealing with difficult situations. The same study group assessed that such a support system would stop using defense mechanisms to alleviate negative experiences.

CONCLUSIONS

The conclusions can be summarized as follows:

1. Most midwives surveyed experienced stress while caring for a patient with a diagnosed fetal defect.
2. Most midwives do not have systemic psychological support for medical personnel in the workplace, and some respondents do not know whether they can receive such systemic support.
3. The education of midwives and the hospital's reference level did not affect the way of coping with stress.
4. Most of the respondents did not participate in courses or training that would be aimed at dealing with difficult situations in the ward.

This research indicates the need to introduce courses and training on topics related to coping with stressful situations into the medical staff training system. Difficult situations in which medical staff meets the suffering and sadness of the patient, burden the mental health of the employees. At the same time, access to systemic support is still insufficient. The information about the lack of trust towards colleagues or superiors at work is disturbing – and the resulting lack of systemic support. Based on the current literature, the scientific community agrees that psychological and emotional support is one of the basic conditions for good management and work organization. Therefore, it is important to realize the need to organize such support in order to improve the quality of work of the ward staff, the quality of services provided, and to obtain job satisfaction.

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ORIGINAL ARTICLE

ACUTE COMPLICATED PANCREATITIS AND DIABETES MELLITUS: THE ROLE AND SIGNIFICANCE OF THE BIOCHEMICAL INDICATORS OF CARBOHYDRATE AND LIPID METABOLISM AS A PROGNOSTIC CRITERION FOR THE SEVERITY OF THE DISEASE CLINICAL COURSE

10.36740/WLek202101104

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ABSTRACT

The aim: A clinical evaluation of biochemical parameters especially carbohydrate and lipid metabolism, their effect on the treatment program in patients with acute complicated pancreatitis.

Material and methods: The results of complex treatment of a sample group of 127 patients with acute pancreatitis were studied. A moderately severe clinical course of the disease was found in 42 patients (33.1%) and severe in 85 patients (66.9%), the ethanol genesis of the disease was stated in 73 (57.5%), biliary genesis – in 54 (42.5%) patients. The evaluation of the treatment effectiveness was based on the analysis of the dynamics of disease clinical manifestations and the monitoring of laboratory biochemical parameters and glucose levels

Results: It was stated, that the clinical course of the disease, severity, and prognosis of the patient's condition correlate with the dynamics of markers of endotoxicosis and hepatic insufficiency, and glucose level is one of the most sensitive criteria. The carbohydrate metabolism dynamics analysis in the blood of patients has shown that in most patients glucose levels increase notably with the increase in pancreatic swelling and reaches a peak by its destruction

Conclusions: The incidence of diabetes mellitus due to primary inflammation of the pancreatic parenchyma in ACP has a linear dependence on the frequency and duration of the disease; with the probability of developing diabetes with an exacerbation of the process during 5-8 years is 17.5%. The level of blood glucose in patients with ACP can be a reliable indicator of the severity of the disease course and correlates with other indicators of metabolism

KEY WORDS: diabetes mellitus, Carbohydrate Metabolism, acute complicated pancreatitis, insulin dependent diabetes

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INTRODUCTION

The epidemiology of secondary diabetes mellitus (DM) in the pathology of the pancreas, in particular in cases with pancreatitis, is not fully investigated. First of all, the reason for the complexity of diagnosis is that clear epidemiological data are not always amenable to statistical and clinical analysis. It is known that short-term hyperglycemia in acute pancreatitis (AP) develops in about half of patients who are hospitalized, and persistent hyperglycemia AP persists with a frequency of up to 15% [1,2,3,4,5].

It should be noted that short-term (transient) hyperglycemia also develops in case of chronic pancreatitis (CP) exacerbation, which in most cases is associated with pancreatic edema and the inhibitory effect of trypsin on insulin production, and its increase in the blood [6,7,8,9,10].

In patients with AP, there is a tendency that is determined by metabolic disorders from carbohydrate metabolism. It was noted that usually in the first day after the disease appearance, blood glucose keeps at the level of reference values, and increases starting from 3-4 days. This is due to numerous stress-gene factors, which are usually affected by the severity of the underlying disease [11].

According to various sources, DM occurs in 15-18% of patients with AP and 10-90% of patients with CP, and in half of cases, insulin-dependent diabetes (IDDM) develops [12]. Such a big difference in the literature data on the incidence of DM with AP and CP is associated both with poor diagnosis of the underlying disease and with a different degree of probability of developing endocrine disorders in various forms of pancreatitis. It should be noted that carbohydrate tolerance is usually observed already in the early stages of observation [7,11,13,14]. DM can also form at the beginning of the clinical manifestation of pancreatitis, but still more often, the persistent disorder of carbohydrate metabolism occurs in about 5 years after the onset of the underlying disease, with chronic inflammation and on the background of frequent remissions of AP (2-3 times a year), when development of DM becomes a linear time dependence [5].

According to some authors [12], in case of the manifestation of the disease (AP and exacerbation of CP), DM is

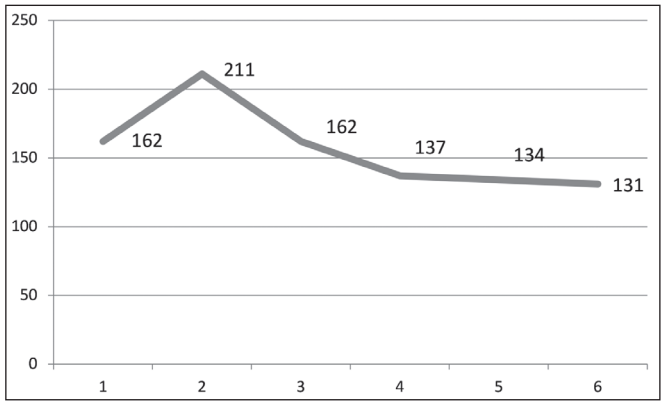

Fig.1. α-amylase level in the blood serum during treatment.

diagnosed in 10% of cases, and in 2% - IDDM. After 10 years from the onset of the underlying disease - in 50 ± 3 and 26 ± 3% of cases. After 25 years, these indicators reach 83 ± 4 and 53 ± 6%. The annual probability of developing DM with pancreatitis of biliary origin is 3.5%, and IDDM - 2.2%. In almost 80-90% of patients whose pancreatic lesions were verified and at least one case in the year of its exacerbation, during 25 years the possibility of DM appearance is 65-85%.

So, despite the fact that the features of the acute complicated pancreatitis (ACP) pathogenesis and clinical course have been widely studied by clinicians in European countries over the last few years, a number of questions remain unresolved [5,9]. This applies, in particular, to the unification of approaches in the clinical assessment of biochemical parameters in patients with ACP, taking into consideration their importance for predicting the course of the disease, the characteristics of the diagnostic algorithm and approaches to conservative treatment [2,3].

THE AIM

The aim of the research, which is coming out of the above mentioned, is to study changes in carbohydrate and lipid metabolism in patients with ACP.

MATERIALS AND METHODS

The results of complex treatment of a sample group of patients with AP in the amount of 127 patients, who were hospitalized in the emergency hospital with a diagnosis of ACP were studied. In accordance with the criteria of Atlanta (2012) [3], a moderately severe clinical course of the disease was found in 42 patients (33.1%) and severe in 85 patients (66.9%). According to the pathogenesis of the disease in 73 (57.5%) patients, the ethanol genesis of the disease was stated, in 54 (42.5%) – biliary genesis.

In all patients, in the complex of conservative treatment, a unified diagnostic treatment algorithm was used, based on local treatment protocols for patients with AP and clinically tested approaches to the management of this patient group. All patients were randomized by age, sex, disease severity, degree of pancreatic lesion, parapancreatic / paracolary retroperitoneal fatty tissue and were treated in the intensive care unit for the first 7-14 days. Surgical treatment (including minimally invasive, puncture techniques) in the case of infected pancreatic necrosis and / or purulent-necrotic parapancreatitis / paracolitis was performed in 74 (58.3%) patients.

Evaluation of the treatment effectiveness was done on the basis of an analysis of the disease clinical manifestations dynamics and laboratory biochemical parameters (carbohydrate, protein, lipid metabolism) monitoring. The obtained results were analysed by using MedStat programe for medical statistics information.

RESULTS

An analysis of the dynamics of laboratory indicators of blood sugar levels (carbohydrate metabolism) in patients with ACP showed that in most patients the blood glucose level increases markedly with an increase in pancreatic edema and reaches its maximum during its destruction.

In the time of hospitalization in almost all patients during first days of the disease, an increase in serum α-amylase levels (N = 30-130 U / L) was detected, which confirmed inflammation of the pancreatic parenchyma - acute pancreatitis (Fig.1).

In 63 patients from the group of examined patients, during the first 12 hours of the disease, the serum glucose content varied within the upper normal range of 5.8 ± 3.1 mmol / L. Subsequently, in all patients, regardless of the pathological process nature and the degree of pancreatic parenchyma damage, an increase in glucose up to the level of 6.3 ± 9.1 mmol / l was observed. In other patients, glucose growth was already observed in the first hours of the disease to levels of 10.4 ± 3.2 mmol / L.

After monitoring changes in serum glucose levels, certain regularity can be noted that allows us to establish a relationship between the degree of glucose growth and the severity of the disease, starting from the second day of treatment of patients with AP. This indicator, according to our observations, is a statistically significant marker for assessing the patient's condition and the effectiveness of the infusion therapy.

The analysis of changes in serum glucose levels allowed us to state a regularity, namely, at the beginning of the disease, glucose levels remain normal or moderate hyperglycemia (5.7 ± 1.3 mmol / l) is stated, and statistically from the second or third day significant ($p<0.05$) increase in blood plasma glucose is observed. This is due to the activation of the insular apparatus functioning in the first hours of the disease and the inhibition of the gland islets activity with the development of destructive changes. In cases with destructive pancreatitis, the insufficiency of the insular apparatus was also stated.

To compose a standard approach to patient's condition assessing, taking into consideration laboratory and instrumental methods and their correlation with general clinical data, we used the following evaluation algorithm. Obser-

Table 1. Dynamics of vital signs during treatment

	Stage of research				
	1 stage	2 stage	3 stage	4 stage	5 stage
RR[1]min.	23,0±0,9*	19,4±0,7	18,9±0,6	17,4±0,5	17,1±0,2*
HR[1], min.	112,7±3,2*	104,6±2,9	94,1±4,0	88,4±3,7*	86,4±4,5*
$SatO_2$,%	95,0±0,7	98,1±0,2	97,7±0,4	97,8±0,2	97,7±0,4
SBP[1]mm.Hg	128,0±9,9*	127,2±9,5	121,1±5,8	117,3±5,8*	124,6±6,3
DBP[1]mm.Hg	79,6±5,2	77,7±5,8	76,4±5,6	76,1±4,2	74,7±4,2
Body temperature.C°	37,8±0,5	38,1±0,4	37,5±0,5	37,1±0,4	37,1±0,2
Diuresis, ml	825±63*	1180±194	1784±112	2045±220	1959±517*

*– the difference is significant compared to the norm (p<0,05)
RR- respiration rate; SatO2 –blood saturation; SBP –systolic blood pressure; DBP – diastolic blood pressure; HR-heart rate

Table 2. Dynamics of biochemistry parameters

Indicator	Stage of research				
	1 stage	2 stage	3 stage	4 stage	5 stage
Total protein, g/l	70,0±0,7*	64,1±0,2	47,5±0,4	40,1±0,2	46,6±0,4*
Glucose, mmol/l	5,86±0,3*	9,13±0,5*	8,71±0,4*	7,50±0,4	9,98±0,7*
Urea mmol/l	8,71±0,3*	10,92±0,2	8,3±0,5	10,8±0,4	14,1±0,3*
Creatininemmol/l	95,0±0,2	121,1±0,7	134,2±0,5	123,4±0,3	113,6±0,4
Bilirubin mmol/l	19,4±0,6*	11,84±0,4	9,0±0,8*	9,3±0,7	18,0±0,6

*–the difference is significant compared to the norm (p<0,05)

Table 3. Dynamics of hematology parameters

Indicator	Stage of research				
	1 stage	2 stage	3 stage	4 stage	5 stage
Hemoglobin, g/l	169,3±0,4*	156,5±0,3	129,3±0,7	112,2±0,2	92,5±0,8*
Leukocytes, 10^9/l	15,0±1,2*	10,2±0,9	14,41±0,1	16,28±0,2	9,1±0,5*
Immature neutrophils, %	5,5±1,4*	4,5±1,1	6,2±1,6	11±1,5*	9,8±0,4*
Lymphocytes, %	21,6±3,2*	17,7±3,6	17,9±2,4	17,1±2,1	15,3±1,9*
Fibrinogen	3,4±0,3	4,8±0,7	4,9±0,6	7,11±0,5	4,8±0,3
Prothrombin index	78,02±0,3	78,11±1,1	85,48±2,4	78,94±1,6	79,15±0,9

*–the difference is significant compared to the norma (p<0,05)

vation of patients was carried out in five stages: 1st – initial stage (hospitalization of the patient); 2nd stage – 1 day after the start of intensive care; 3rd, 4th and 5th – the corresponding day of the disease (assessment of the patient's condition and the effectiveness of drug therapy). Descriptive statistics methods were used to describe the complex of primary data. The difference was considered significant and statistically significant at p <0.05. (Tab.I)

At the time of patients hospitalization, the mean systolic arterial pressure (SAP) was (128.0 ± 9.9) mmHg, with severe tachycardia (112.7 ± 3.2) min. and tachypnea (23.0 ± 0.9) min. By the end of the second day, as a result of the therapy, the majority of patients showed a tendency to a decrease in the SAP to the levels of (121.1 ± 5.8) mm Hg, which was largely due to the conducted infusion therapy.

The normalization of SatO2 is also noteworthy, which is largely due to the leveling of stress-gene factors (pain, providing the body with energy donors, correction of metabolic disorders, support for active protein mass, prevention of background immunosuppression).

According to the data presented in table 2, it is possible to draw preliminary conclusions that in patients with AP, signs of intoxication tend to increase. According to the results of biochemical studies, an increase in the level of urea was observed from 8.71 ± 0.3 to 14.1 ± 0.3 mmol / L on the background of progressive hypoproteinemia (at the time of hospitalization, 70.0 ± 0.7 g / L), while on the fifth day of treatment, the level of blood proteins decreased to levels of 46.6 ± 0.4 g / l. (Tab. II)

According to peripheral blood examination results during 1-3 day of treatment, the signs of dehydration (hemoglobin 169.3 ± 0.4 g / l) with a reliable (p<0.05) with a

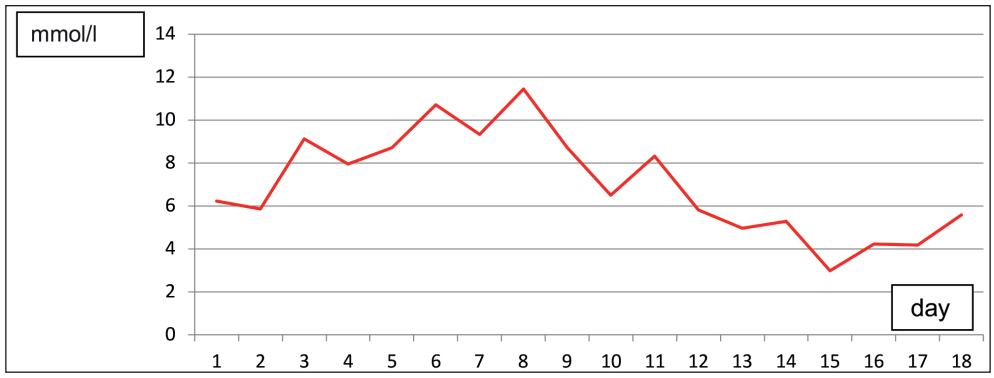

Fig. 2. Dynamics of glucose levels.

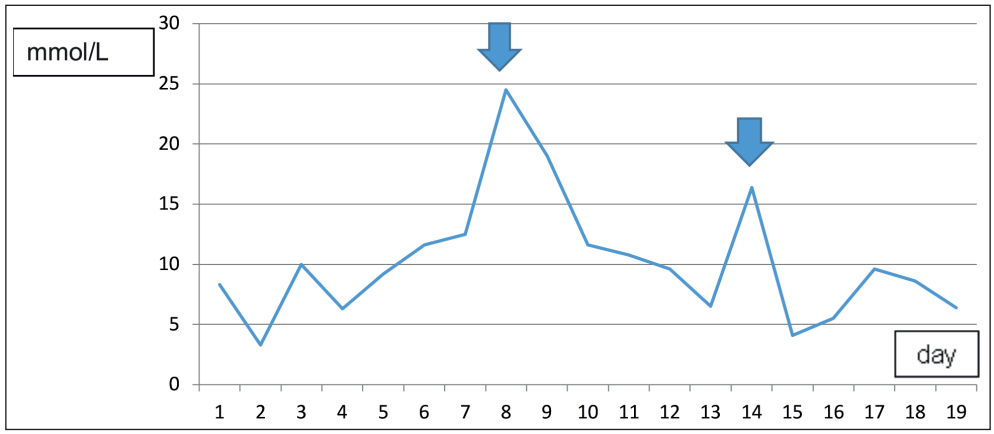

Fig.3. Dynamics of glucose levels according surgery treatment.

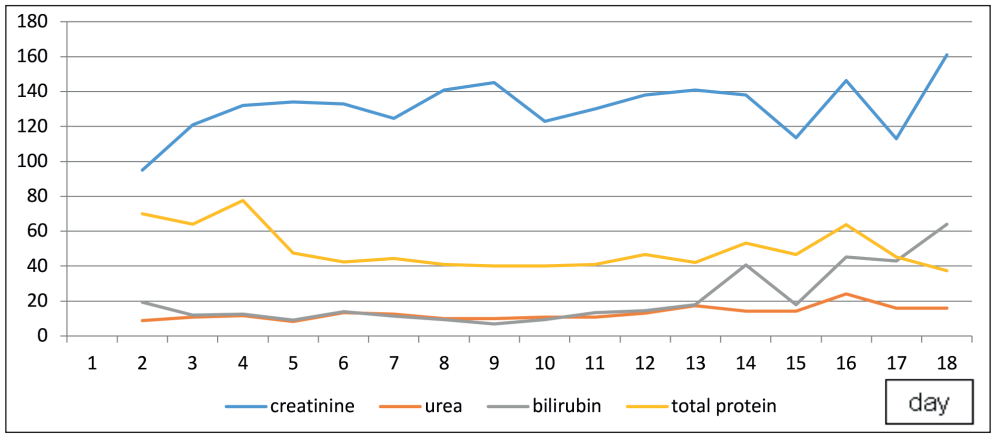

Fig.4. Dynamics of biochemical parameters.

tendency to its level decrease during treatment were stated (92.5 ± 0.8 g / l). The majority of patients had confirmed signs of a systemic inflammatory reaction: a quantitative increase in the leukocytes level (16.28 ± 0.2 x 109 / L) in peripheral blood and stab neutrophils (11.0 ± 1.5%) on the background of a lymphocytes level decrease (15.3 ± 1.9%).(Tab. III)

Comparison of blood glucose levels changes at different periods of the disease with insulin content allowed us to make a conclusion that the glucose level depends on the severity of the clinical course of ACP and the function of the insulin apparatus of the pancreas, and therefore can serve as a marker of the disease clinical course severity. The dynamics of glucose levels changes in the peripheral blood serum during treatment (in days) is shown in Fig..2. The peak increase in blood glucose levels, as a rule, coincided with surgical interventions (mini-invasive and / or open surgery), which served as a kind of stress-generating factors for the patient's body in ACP (Fig.3).

In the course of analyzing the patient's condition during treatment, we monitored laboratory indicators of metabolic disorders, which, although not being triggers in the development of acute pancreatitis, however, with its further development, they become important in pathogenesis and directly determine the clinical course and consequences of the disease. During the study, we monitored the biochemical parameters (total protein and its fractions in the blood, the level of urea, creatinine, bilirubin) - (Fig.4).

Undoubtedly, the assessment of biochemical parameters in the general plan of the ACP treatment algorithm

allowed us to determine the depth and direction of metabolic disorders, the mechanism for the development of other changes in the patient's body, and also made it possible to assess the nature of pathological changes in the pancreas. However, it is extremely difficult to clearly state that these indicators are the criteria for determining the clinical course of ACP. It should be noted that we did not find a clear correlation between these indicators in the group of examined patients, although indirectly they characterized the patient's condition and the clinical course of the disease.

In cases with severe gland edema, a moderate increase in the respiration rate (18 ± 2.1) min was detected, which is directly related to the processes of phosphorylation and the rate of ATP production. Hemorrhagic pancreatitis was accompanied by an increase in respiratory rate in active and controlled states (20 ± 3.4) min. on the background of intensification of ATP production, and with necrotic pancreatitis, a more pronounced increase in respiratory activity is characteristic (respiratory rate up to 24 ± 2.8 min.). This is usually due to the fact that hemorrhagic pancreatitis is accompanied by an increase in energy production mainly due to intensification of respiration, and with necrotic forms of the disease, a relative depression of bioenergetic processes in liver mitochondria was observed.

DISCUSSION

According to the abovementioned data, DM does not develop in all patients with AP. In acute lesions of the pancreatic gland, islet cells are often well preserved despite severe damage to acinar cells [8]. The mechanism of the relative conservation of endocrine cells compared with the destruction of exocrine parenchyma does not have a clear explanation, but may be associated with a predominant increase in the apoptosis index in acinar cells compared to islet cells. Probably due to the mechanism of blocking apoptosis [4,8].

The pathogenesis of pancreatogenic diabetes is mainly explained by the development and progression of destructive changes, hypoxic changes in devitalized pancreatic tissues, and endocrine sclerosis. However, an autoimmune effect on islet cells is also possible, which correlates with morphological and functional changes in β-cells [4,8].

A certain role in the pathogenesis of diabetes in ACP is played by constitutionally determined tissue resistance, which is much more common in individuals with obesity and hyperlipidemia [14,15]. Obesity aggravates the clinical course of the disease and complicates its prognosis.

In general, according to the results of the study, it can be noted that the clinical course of the disease, severity and prognosis of the patient's condition correlate with the dynamics of endotoxemia markers and liver failure, and among the general indicators of protein and carbohydrate metabolism, sugar level is one of the most sensitive criteria.

Thus, the determination and monitoring of the blood glucose level in patients with ACP allows early identification of the severity of the process and predicts a possible adverse course, adjusts the starting therapy, identifies the need for indications for surgical intervention and in cases of high risk of an adverse outcome, prescribes enhanced antibacterial and detoxification therapy.

CONCLUSIONS

1. The frequency of diabetes mellitus development as a result of primary inflammation of the pancreatic parenchyma in ACP has a linear dependence on the frequency and duration of the disease, and the likelihood of developing diabetes with an exacerbation of the process during 5-8 years is 17.5%.
2. The pathogenesis of pancreatogenic diabetes mellitus is associated with the destruction and sclerosis of endocrine pancreatic tissue, and in the presence of obesity, with insulin resistance.
3. Blood sugar levels in patients with ACP can be a reliable indicator of the severity of the disease course and correlates with other indicators of metabolism.
4. The incidence of diabetes with alcohol pancreatitis is twice higher than with the biliary genesis of the disease.

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ORIGINAL ARTICLE

FETAL INFECTIONS OPTIMIZATION OF PREGNANCY AND DELIVERY INTRODUCTION

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ABSTRACT

The aim: Improving perinatal outcomes in pregnant women at high risk of intrauterine infection by developing diagnostic criteria and algorithms for managing pregnancy and childbirth.

Materials and methods: The study of pregnancy and childbirth was conducted in 72 patients at high risk of IUI, which formed the main group. The control group consisted of 64 patients with a low infectious risk of IUI. Culture, bacterioscopic and biochemical methods were used to identify microorganisms. Peculiarities of infection in the examined women were investigated by determining the concentration of Ig M and IgG in the blood serum and performed polymerase chain reaction for measles virus, cytomegalovirus, parvovirus B19. Serum for the presence of specific immune globulins to these pathogens was examined by ELISA. Comprehensive ultrasound examination in B-mode was performed to determine the feto metry of the fetus and assess its development with the determination of the estimated mass, location, size and structure of the placenta, the amount of amniotic fluid. To determine the condition of the fetus, a Doppler study of blood flow in the uterine arteries, umbilical artery, middle cerebral artery of the fetus and venous duct.

Results: Analyzing the course of this pregnancy in women of the studied groups threatened miscarriage and the threat of premature birth occurred in 24 (33.3%) cases, with signs of isthmic-cervical insufficiency were diagnosed in 13 (18.1%) patients. In the control group of patients, the threat of abortion was diagnosed in 15 (23.4%) patients. According to ultrasound examination, patients in the main group in 12 (16.7%) cases were diagnosed with fetal growth retardation, in 25 (34.7%) patients at high risk of IUI there were changes in the placenta, namely, hyper echogenic inclusions in the placenta occurred in 7 (9.7%) cases, dilation of the intervillous space in 8 (11.1%) cases, placental hyperplasia in 7 (9.7%) cases, polyhydramnios was diagnosed in only 5 (6), 9%) cases, with 1 (1.4%) acute polyhydramnios in patients with signs of acute respiratory viral infection during pregnancy.

Conclusions: Women at high risk for IUI require close monitoring of the fetus due to the increased frequency of hemodynamic changes in uteroplacental-fetal circulation, including fetal-placental – 22.2% and the occurrence of intrauterine growth retardation.

Women with suspected cytomegalovirus infection require determination of seroconversion; in case of immunologically confirmed infection, it is desirable to recognize PCR for cytomegalovirus in the amniotic fluid in order to determine further management and monitoring of this pregnancy.

KEY WORDS: fetal growth retardation, antibiotic prophylaxis, miscarriage, inflammatory diseases, cytomegalovirus infection

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INTRODUCTION

Recently, more and more attention has been paid to increasing the frequency of intrauterine infection (IUI) in developing the perinatal pathology, in particular in the emergence of fetus critical condition [1,5,9,16]. In most countries, the registered increase in the role of IUI in forming the pathology of the perinatal period and the situation with IUI is equated to an epidemic. One of the most urgent tasks of perinatology at present is the problem of predicting fetal IUI using methods available in modern obstetrics. Despite the fact that many works are devoted to this problem, so far there is no holistic view of the course of pregnancy, fetal development and the likelihood of its intrauterine infection when infecting the mother. There are no common views on the pathogenesis, prevention, treatment of intrauterine infections, obstetric tactics in IUI [2, 3, 8, 17, 20].

A typical fetal response to infection is developing the fetal inflammatory response syndrome, which is difficult to verify in the fetus and can only be determined postnatally by immunological studies in the newborn (determination of cytokines and chemokines such as interleukins, C-reactive protein and matrix metoloproteinase) [4, 6, 9, 11]. The cytomegalovirus infection (CMV) is known to be the most common cause of neurosensory hearing loss and mental retardation 0.2 – 2.2% of all newborns, 10-15% of congenitally infected children will have symptoms at birth: fetal growth retardation, microcephaly, hepatosplenomegaly, petechiae, jaundice of newborns, chorioretinitis, thrombocytopenia and anemia. Most congenitally infected children (85-90%) do not have any signs of the disease after birth, but 5% – 15% of them will later develop complications such as neurosensory deafness, psychomotor developmental

delay and visual impairment [6, 13, 17, 19]. Parvovirus-B19 infection is a common cause of fetal anemia in the second trimester of pregnancy. This necessitates intrauterine replacement blood transfusion [14, 15, and 17]. The risk of developing fetal chickenpox syndrome is 1-2% with infection during pregnancy up to 20 weeks and is manifested by neurological disorders (mental retardation, microcephaly, hydrocephalus, convulsions, Horner's syndrome), ocular abnormalities, optic nerve atrophy, microphthalmia, catarrh, nystagmus), abnormalities in the development of the extremities (atrophy, paresis, hypoplasia), gastrointestinal disorders (gastro esophageal reflux, intestinal atresia or stenosis) [1, 7, 10]. Group B *Streptococcus*, or *Streptococcus* agalactiae, are gram-positive bacteria that cause infectious disease primarily in newborns, but also in pregnant women, postpartum women (uterine sub involution and early acute pyelonephritis). Early onset of the disease in newborns (during the first week of life): respiratory distress, apnea, or other signs of sepsis during the first 24-48 hours of life. The most common clinical syndrome of the disease with an early onset is sepsis and pneumonia, rarely meningitis. There is mortality of the disease with early onset up to 50% without treatment; during therapy – 4 - 6%. Mortality is higher among premature infants, at about 20%. Infection of the fetus occurs intranatally in pregnant women who are carriers of group B *streptococcus* [14, 16, and 19].

The mechanism of developing the intrauterine infection is quite complex and many aspects of this problem are still debatable, need further study [20, 21, 22]. A significant part of diseases in pregnant women, which lead to intrauterine infection of the fetus, occurs in subclinical or latent, asymptomatic form, have no characteristic clinical manifestations, which significantly complicates the diagnosis of this pathology in the antenatal period [2, 3, 7, 23].

So, contradictory data on the informativeness and diagnostic value of various methods for predicting the occurrence of IUI, as the reasons for developing the critical state of the fetus and newborn, indicate the need to systematize the data and conduct a comparative analysis. Searching and developing the algorithm of diagnostics, forecasting the emergence of IUI and its prevention by methods available in practical obstetrics, estimating the informativeness of the received results is an actual task of modern perinatology [7, 9, and 12].

THE AIM

Improving perinatal outcomes in pregnant women at high risk of intrauterine infection by developing diagnostic criteria and algorithms for managing pregnancy and childbirth.

MATERIALS AND METHODS

To achieve this goal, a comprehensive study of pregnancy and childbirth was conducted in 72 patients at high risk of IUI, who were registered and treated in the city clinical maternity hospital №2 in Kyiv, which formed the main group. Criteria for inclusion of patients in the study were: the presence of a children's birth history or fetuses with signs of intrauterine infection, confirmed clinically and/or morphologically. The control group consisted of 64 patients with a low infectious risk of IUI.

Clinical examination of patients was performed on a specially designed questionnaire, which included primarily a detailed collection of anamnestic data to identify possible risk factors for IUI. The analysis of pregnancy consisted of collecting data on the course of gestation, data of additional instrumental and laboratory methods of research, in particular data of bacterioscopic, bacteriological study of material from the vagina and urine, enzyme-linked immunosorbent assay for perinatal infections (TORCH – infections).

To study the condition of the vaginal microbiocenosis in women of certain groups, a bacterioscopic study of vaginal contents with Gram staining was performed, followed by microscopy with an immersion lens. Bacteriological examination of the contents of the vagina and urine was performed by inoculating the material on nutrient media: 5% blood agar, sugar broth, Endo medium. The crops were incubated at a temperature of 37° C, viewed daily. When growth appeared on dense media, colonies of different morphology were counted, taking into account their ratio. A negative result of the study was found in the absence of growth on all nutrient media for 72 hours. Culture, bacterioscopic and biochemical methods were used to identify microorganisms. Characterization of the vagina biocenosis was performed according to the criteria of E.F. Kira.

Peculiarities of infection in the examined women were investigated by determining the concentration of Ig M and IgG in the blood serum and performed polymerase chain reaction (PCR) for measles virus, cytomegalovirus, parvovirus B19. Serum for the presence of specific immune globulins to these pathogens was examined by ELISA. Enzyme-linked immunosorbent assay systems from Novum diagnostic (Germany) and Strip enzyme-linked immunosorbent assay (Stat fax-300) (USA) were used at a wavelength of 450 nm. Luminescent microscopes ML-2A, LUMAM-II were used to detect specific pathogen antigens by polymerase chain reaction in scrapings of the cervical canal. The method allowed not only detecting the presence of specific hypertension in epithelial cells, but also its typical localization in the structural elements of cells.

Comprehensive ultrasound examination in B-mode was performed to determine the feto metry of the fetus and assess its development with the determination of the estimated mass, location, size and structure of the placenta, the amount of amniotic fluid. To determine the condition of the fetus, a Doppler study of blood flow in the uterine arteries, umbilical artery, middle cerebral artery of the fetus and venous duct (extra placental and fetal hemodynamics) (PHILIPS ATL-HDI 4000, PHILIPS HD 11-XE) was performed. The state of peripheral vascular resistance was assessed by determining the indices - SDR (systolic-diastolic ratio), PI (pulsation index), and IR (resistance index) with the calculation of the cerebra-placental ratio.

Variable-statistical processing of the research results

was performed using the program "Statistica 6.0" with the definition of the main variable indicators: mean values (M), mean errors (m), standard deviations (p). The reliability of the results was determined using the Student's t test.

RESULTS AND DISCUSSION

The age, social and marital status of the group of women did not differ significantly. Analyzing the anamnestic data of women in the main group, it was found that 8 (11.1%) patients had births in previous pregnancies with congenital conjunctivitis, 15 (20.8%) – with congenital sepsis, in 39 (54.2 %) – with congenital pneumonia, in 1 (1.4%) – with congenital carditis, in 5 (6.9%) – with congenital dacryocystitis and in 4 (5.6%).

Regarding the data of somatic anamnesis, women of the main group had a burdened somatic anamnesis in 58 (80.6%) patients, which was statistically significantly more than in women of the control group – 24 (37.5%) cases. So, in women of the main group, diseases of the urinary system (chronic pyelonephritis, urolithiasis) occurred in 35 (48.6%) cases, respiratory – in 22 (30.6%) cases, from the organs of the gastrointestinal tract – in 15 (20.8%) patients and LOR-organs – in 5 (6.9%) pregnant women. In the control group, chronic pyelonephritis occurred in the anamnesis of 5 (7.8%) pregnant women, urolithiasis – in 4 (6.3%) patients, chronic gastritis occurred in 7 (10.9%) patients and LOR-pathology – in 2 (3.1%). The exacerbation of chronic pathology during pregnancy in women of the main group was observed in 49 (68.1%) cases, while in the control group – in 5 (7.8%). It should be noted that 15 (20.8%) patients of the main group and 7 (10.9%) pregnant women of the control group had manifestations of acute respiratory viral infection during pregnancy of varying severity, which required additional examination and monitoring. In the main group of women, a burdensome obstetric and gynecological history was probably more common: inflammatory diseases of the uterus and appendages were observed in 35 (48.6%) patients, infertility – in 6 (8.3%), functional ovarian cysts and menstrual disorders occurred in 8 (11.1%) and 6 (8.3%) cases, respectively, genital herpes – in 2 (2.8%) women, abortion – in 28 (38.9%), spontaneous abortion – in 6 (8.3%) and frozen pregnancy – in 4 (5.6%) patients. In the control group, inflammatory diseases of the uterus and appendages occurred in 4 (6.2%) cases, abortion – in 8 (12.5%).

Thus, women at high risk of IUI have a burdensome somatic history with exacerbation during pregnancy, which according to the literature is associated with the development of obstetric pathology and the risk of intrauterine infection. In addition, according to the data, women who gave birth to children with IUI were 7 times more likely to have a burdensome obstetric and gynecological history, especially inflammatory diseases, including specific etiology, a history of chlamydia diagnosed in 5 (6.9%) patients, trichomoniasis – in 13 (18.0%), genital mycoplasma – in 4 (5.6%) cases).

Analyzing the course of this pregnancy in women of the studied groups threatened miscarriage and the threat of premature birth occurred in 24 (33.3%) cases, with signs of isthmic-cervical insufficiency with cervicometry data were diagnosed in 13 (18.1%) patients, which in 1 (1.4%) of the case required the imposition of a cervical suture on the cervix, taking into account the anamnestic data with preliminary preparation and examination. In the control group of patients, the threat of abortion was diagnosed in 15 (23.4%) patients. Examining and managing the women of these groups was carried out according to the order № 417 of the Ukraine Ministry of Health, which requires mandatory microscopic and microbiological examination of vaginal secretions and urine to diagnose bacterial vaginosis and asymptomatic bacteriuria as risk factors for obstetric complications and intrauterine infection. Thus, the normocenosis and the intermediate type of vaginal biocenosis, which is considered a variant of the norm, in women of the main group was found in 15 (20.8%) cases, bacterial vaginosis was diagnosed in 41 (56.9%) cases and nonspecific colpitis in 16 (22.3). %) cases. In the control group of patients, bacterial vaginosis was diagnosed in 13 (20.3%) cases and non-specific colpitis in 9 (14.1%), which required appropriate correction according to the diagnosed changes. Regarding the culture of urine, in patients at risk of IUI in 49 (68.1%) cases there was asymptomatic bacteriuria, while hemolytic group B *streptococcus* was diagnosed at a concentration of 10^5 or more CFU in 21 (29.2%) pregnant women, which required appropriate treatment during pregnancy and antibiotic prophylaxis of infection during childbirth, in 19 (26.4%) cases diagnosed with group B *streptococcus* and urine at a concentration of less than 105, which did not require treatment during pregnancy, but was an indication of mandatory infection prevention in childbirth. Regarding the control group, 12 (18.7%) patients were diagnosed with asymptomatic bacteriuria, and only in 1 (1.6%) case there was group B *streptococcus* in a concentration of less than 10^5 (Figure 1).

Separate observation and examination were required for patients with signs of acute respiratory viral disease during pregnancy with various clinical manifestations and severity – 15 (20.8%) patients of the main group and 7 (10.9%) pregnant women in the control group. Thus, fever and general malaise disturbed all women in the groups with acute respiratory viral infection – 22 (31.7%), while intrauterine pneumonia was diagnosed in 9 (12.9%) patients, rash occurred in 5 (7.2%) pregnant women, myalgia – in 4 (5.8%) women. Given the clinical course, these pregnant women were offered a test for cytomegalovirus infection (Figure 2), parvovirus infection and measles examination (Figure 3) (taking into account the epidemic circumstances at the time of the disease).

Therefore, after determining seroconversion in women with clinical symptoms of acute viral infection in 3 (4.3%) cases diagnosed with Ig M and Ig G with low avidity to cytomegalovirus, which may indicate a high risk of fetal infection. When conducting ultrasound examination, one patient was diagnosed with ultrasound markers of infection, namely, hepatospenomegaly, for which, to clarify the diagno-

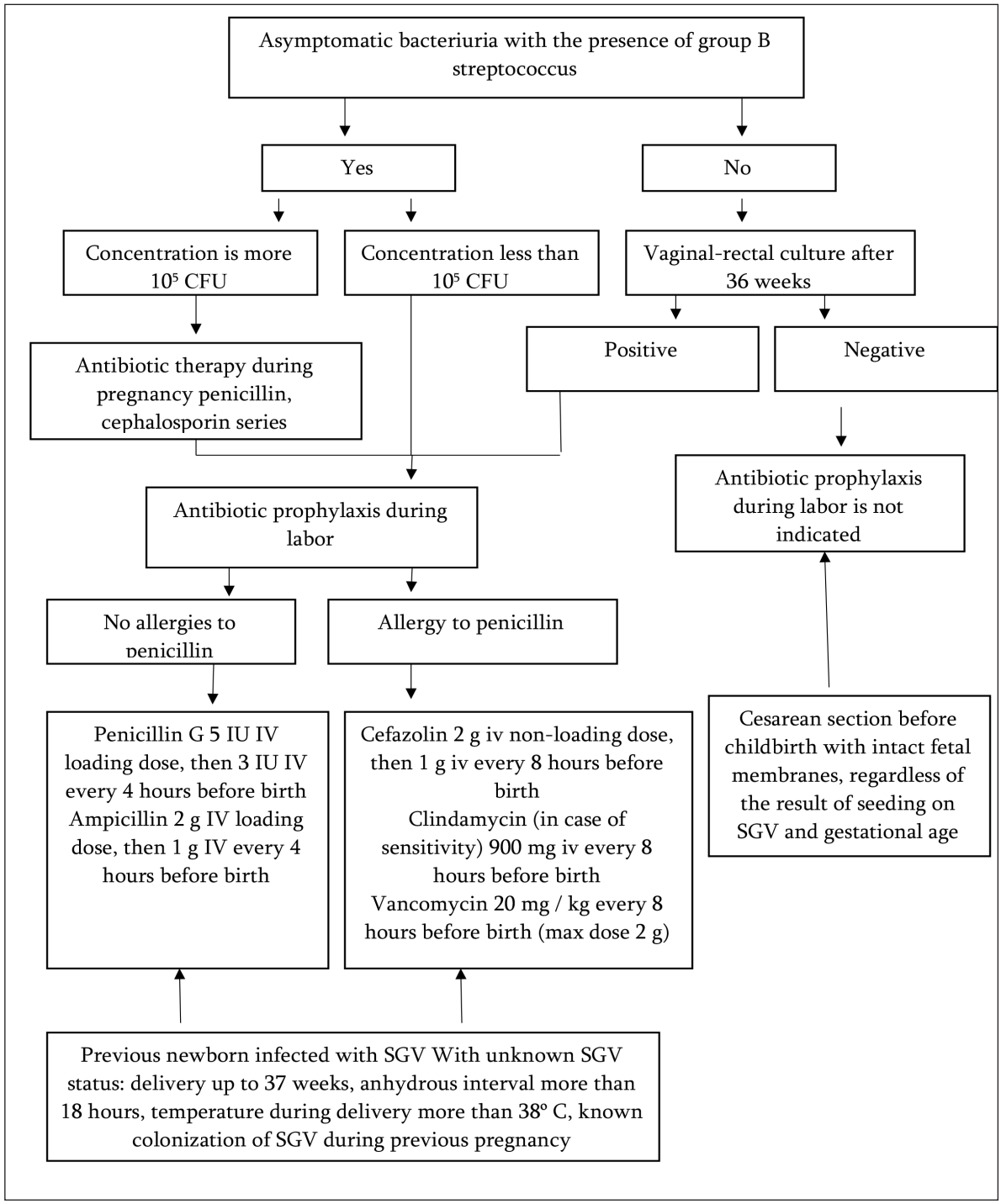

Fig. 1. Algorithm of management of women - carriers of group B *streptococcus*.

sis and further tactics of managing a pregnant woman, it was proposed to conduct amniocentesis with the determination of cytomegalovirus by the polymerase chain reaction (PCR) method. In 4 (5.8%) patients after the analysis of the epidemic anamnesis examination for measles was performed, taking into account the presence of contact with the patient. In 2 cases, in the absence of Ig G, specific immunoglobulin was administered with repeated Ig G control after 3 weeks.

According to ultrasound examination, patients in the main group in 12 (16.7%) cases were diagnosed with fetal growth retardation, which required special monitoring during this pregnancy, in 25 (34.7%) patients at high risk of IUI there were changes in the placenta, namely, hyper echogenic inclusions in the placenta occurred in 7 (9.7%) cases, dilation of the intervillous space in 8 (11.1%) cases, placental hyperplasia in 7 (9.7%) cases, polyhydramnios was diagnosed in only 5 (6) , 9%) cases, with 1 (1.4%) acute polyhydramnios in patients with signs of SARS during pregnancy. At the same time, ultrasound changes in placenta and water volume did

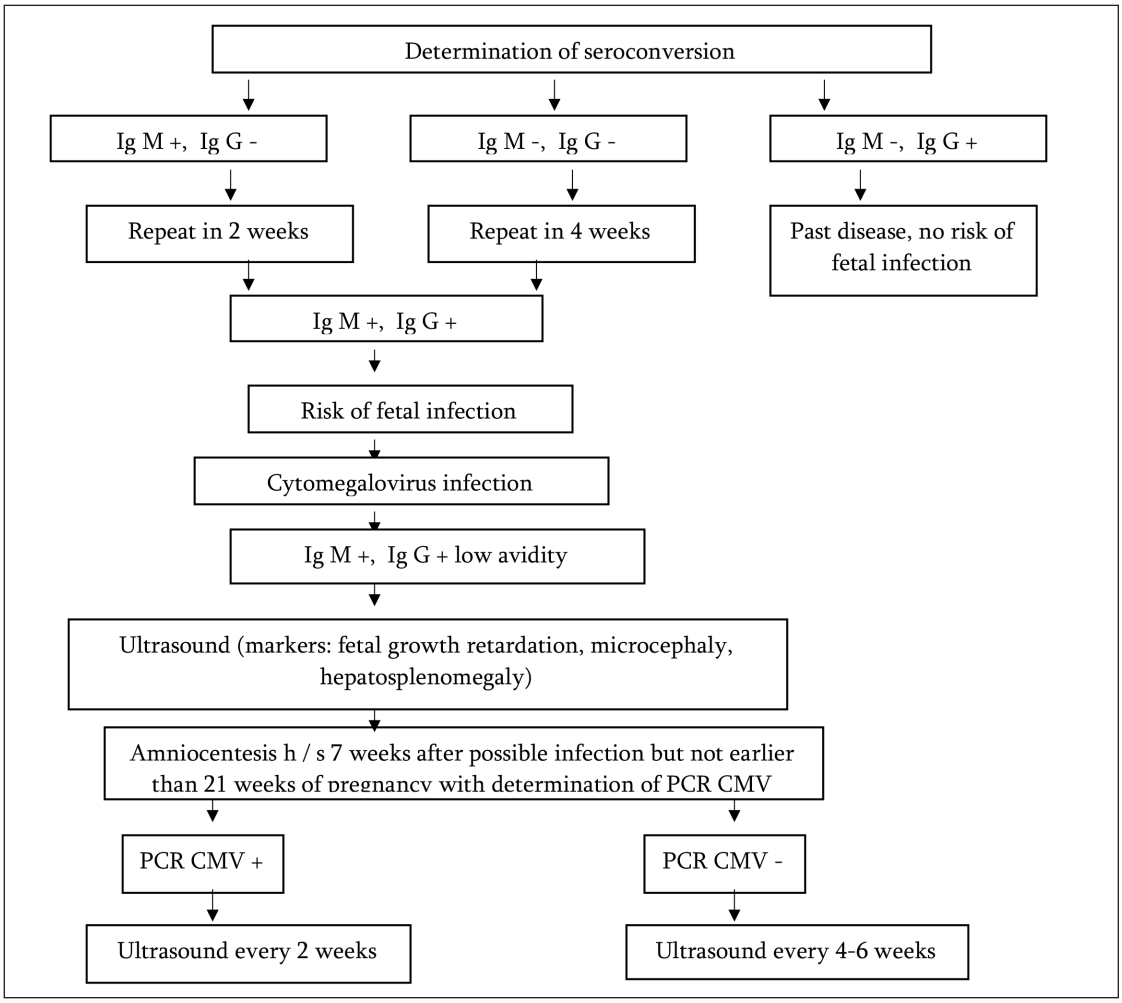

Fig. 2. Algorithm for managing women with CMV infection.

not have a statistically significant difference in women in the control group, except for the percentage of cases with fetal growth retardation, which were not in women at low risk of IUI.

Investigating the hemodynamic characteristics of the uterine-placental-fetal complex, an increase in the resistance index in the uterine arteries in women of the main group from 0.53 ± 0.03 to 0.68 ± 0.06, in the control – from 0.48 ± 0.02 to 0, 95 ± 0.19 due to the slowing of diastolic blood flow, also applied to the pulsation index – 0.97 ± 0.02 in women at increased risk of IUI. Hemodynamics in the umbilical artery in women of the main group was characterized by an increase in IR of 0.71 ± 0.02, in the control – from 0.61 ± 0.01, PI – 0.97 ± 0.03 and 0.89 ± 0.05, respectively, indicating a violation of placental and fetal blood flow at high risk of IUI. Thus, dopplerometry in women of the main group revealed a violation of uteroplacental blood flow in 4 (5.6%) cases, fetal-placental – in 16 (22.2%) and uteroplacental-fetal blood flow – in 5 (6.9) %) cases, in women of the control group there was a violation of uteroplacental blood flow in 2 (3.1%) cases.

Regarding the course of childbirth, in 53 (73.6%) cases in women of the main group childbirth was urgent, premature birth took place in 19 (26.4%) cases, with only 5 (6.9%) patients there was a need premature birth due to anenatal fetal distress and in 14 (19.5%) there was a premature rupture of membranes (PRPO). In the control group of women, premature births occurred in 8 (12.5%) cases, in 4 (6.2%) on the background of PRPO. The course of preterm labor in women of both groups was statistically significantly different. Thus, in women with high risk of IUI there was PRPO in 6 (8.3%) cases and in the control group – in 4 (6.2%), weakness of labor developed in 10 (13.9%) patients of the main group and in 8 (12.5%) in the control. 15 (20.8%) women in the main group gave birth by cesarean section, of which 4 (5.5%) from fetal growth retardation and 11 (17.2%) from the control group.

The average weight of newborns in women at high risk of IUI was – 3344.0 ± 101.0, height 51 ± 4 cm, in the control 3540.2 ± 119.1, height 52 ± 3 cm. The Apgar score of the newborns of the main group at the first minute was 7.8 ± 0.5 points, at the fifth minute – 8.3 ± 0.5 points and there was no significant difference with the newborns of the control group (7.9 ± 0.5 and 8.6 ± 0.4, respectively). Most newborns did not need primary resuscitation in the delivery room. In women of the main group, 4 (5.5%) children were born in severe asphyxia; they were born prematurely due to Antenatal distress against the background of fetal growth retardation; in a state of moderate asphyxia, 5 (6.9%) children were born with mild asphyxia – 5 (6.9%).

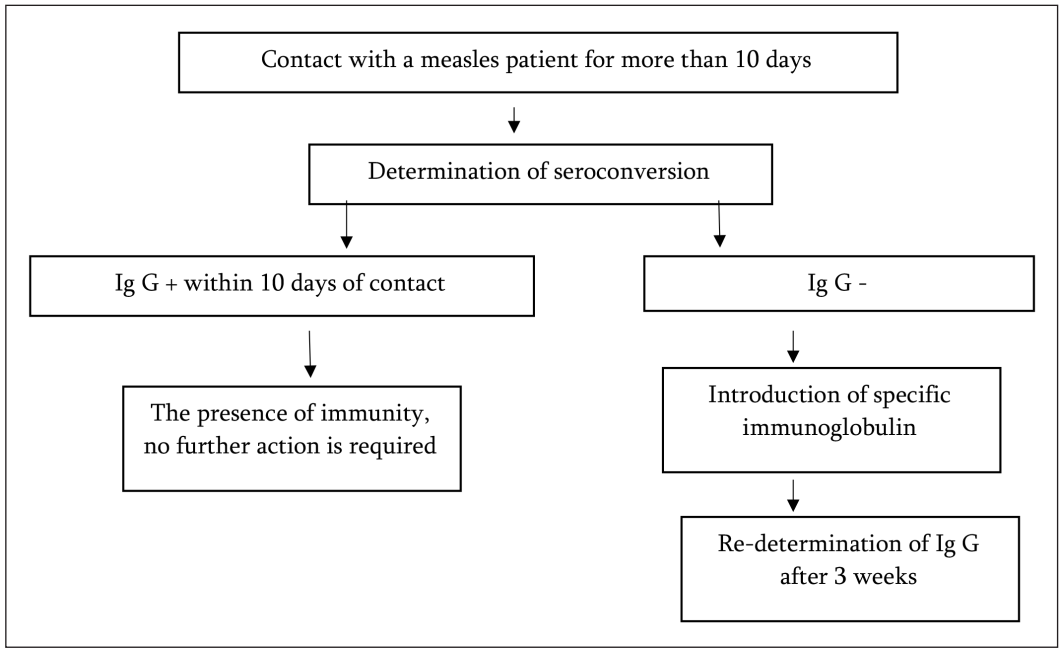

Fig. 3. Algorithm for managing women with suspected measles.

Regarding children in the control group, the average and mild degree of asphyxia at birth occurred in 3 (4.8%) and 4 (6.2%) cases, respectively, which was statistically significantly different. In children with severe asphyxia, neurological symptoms occurred in the form of moderate depression of the central nervous system, increased neuro reflex excitability. In 3 (4.2%) children of the main group there was a cephalohematoma, which may be associated with IUI, in 4 (5.6%) hemorrhagic syndrome in the form of skin and hemorrhagic manifestations, toxic erythema with small papular rash on the trunk and extremities, which disappeared on its own 4-7 days after birth.

CONCLUSIONS

1. Thus, as a result of the study, risk factors for intrauterine infection were identified, namely, burdened obstetric and gynecological history (artificial abortions – 48.6%, inflammatory diseases of the uterus and appendages – 38.9%), burdened somatic history – 80.6% (diseases of the urinary system – 48.6%, respiratory system – 30.6%, gastrointestinal tract – 20.8%, manifestations of acute respiratory viral infection during pregnancy – 31.7%, which requires additional examination for a specific infectious process with appropriate monitoring of this pregnancy).
2. Women at high risk for IUI require close monitoring of the fetus due to the increased frequency of hemodynamic changes in uteroplacental-fetal circulation, including fetal-placental – 22.2% and the occurrence of intrauterine growth retardation.
3. Carriers of group B *streptococcus* – 55.6% is an important risk factor for IUI and requires mandatory treatment during pregnancy at urinary concentrations of more than 10^5 CFU with subsequent antibiotic prophylaxis in childbirth, women with no culture of *streptococcus* B in the urine need screening for its carrier by vaginal-rectal culture at 36 weeks of pregnancy.
4. Women with an undetermined status regarding group B *streptococcus* in case of delivery earlier than 37 weeks, with an anhydrous interval of more than 18 hours, an increase in body temperature during childbirth above 38° C, at birth of a child infected with group B *streptococcus* during previous vaginas, need mandatory antibiotic prophylaxis during childbirth.
5. Women with suspected cytomegalovirus infection require determination of seroconversion; in case of immunologically confirmed infection, it is desirable to recognize PCR for cytomegalovirus in the amniotic fluid in order to determine further management and monitoring of this pregnancy.

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ORIGINAL ARTICLE

PROGNOSTIC SIGNIFICANCE OF MAGNETIC RESONANCE IMAGING IN PATIENTS WITH PROSTATE INTRAEPITHELIAL NEOPLASIA

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ABSTRACT

The aim: To determine prognostic significance of mpMRI in prostate intraepithelial neoplasia (PIN) diagnostics.

Materials and methods: The results of examination of 52 patients with PIN were assessed in mpMRI using PIRADS criteria. The total number of samples with PIN amounted 166. According to PIRADS MRI assessment of central and peripherial zones was made separately. The use of T2WI, DWI, DCE in patients with high grade and low grade PIN was studied. MRI was performed before prostate biopsy (MRI cognitive fusion biopsy). During 3-year follow-up rebiopsies were performed with prostate cancer detection. PIRADS values of PIN lesions with malignisation were compared with those without following tumor transformation.

Results: There was a difference in values of PIRADS characteristics between PIN and benign prostatic tissue. The mean of PIRADS gradation in samples with PIN was 2,1. Among them 47 (28,3 %) PIN samples had gradation 3 (the presence of clinically significant cancer is equivocal), in 8 (4,8 %) cases – gradation 4 (clinically significant cancer is likely to be present). The mean of PIRADS gradation was in 24 % larger in cases with subsequent PC detection than in cases without malignisation.

Conclusions: MRI parameters in PIN cases differ from normal prostate tissue. PIRADS assessment has prognostic significance of following malignisation of PIN pieces that have similar properties on MRI as prostate cancer. Further study is required to stratify all PIN patients into groups of high malignisation risk in order to perform detailed examination and treatment.

KEY WORDS: prostate intraepithelial neoplasia, multiparametric MRI

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INTRODUCTION

Prostate intraepithelial neoplasia (PIN) is considered to be a precancerous lesion and is of great medical and social importance. According to WHO (world health organization) data the incidence of prostate cancer (PC) in the world is about 1100000 new cases, besides PC mortality is about 300000 [1]. One of the ways to improve early diagnostics and treatment results is timely detection of precancerous processes, their study and development of diagnostics, treatment and prognosis algorithm in patients with precancerous diseases [2,3]. Such high informative method as magnetic resonance imaging (MRI) for a long time is used in PC diagnostics. Technical improvement of MRI with use of multiparametric methods has improved its sensitivity and specificity [4].

Structural MRI parameters charactirise anatomic peculiarities (T2), that are combined with functional parameters (DWI – diffusion-weighted imaging, contrast inhancement) to detect biological tumour properties [5]. Using together these methods allow to assess simultaneously different tissue properties, to determine zonal prostate anatomy and to define vascularisation rate of prostate neoplasms [6,7].

At the same time diagnostic role of MRI in patients with PIN is determined insufficiently. Considering clinical significance of PIN as a precancerous state it is actual to define specific MRI features of PIN for early diagnostics and prevention of malignisation [8]. Systematization and instrumental data comparison are essential requirements for detection of specific MRI properties of precancerous processes. Prognosis of clinical course of PIN and its malignant potential is based on pathomorphology and immunohystochemistry data. The use of mpMRI for determination of high malignisation risk group among patients with PIN aims to further detailed examination of patients with rebiopsy and special treatment.

THE AIM

To study informativeness of mpMRI in prostate intraepithelial neoplasia diagnostics and prognostication of malignant transformation.

MATERIALS AND METHODS

We studied 52 patients from 55 to 73 years old (mean age 66±2,3 y.o.) who underwent transrectal multifocal prostate

biopsy under trasrectal ultrasonic guidance diagnosing PIN. 34 patients had peripheral zone PIN, 11 patients had central zone PIN and 7 patients had both peripheral and central zone PIN. MRI was perfomed before prostate biopsy. Indication to biopsy was PC suspicion based on PSA, digital rectal examination, ultrasonography. Then cognitive fusion MRI targeted biopsy was perfomed.

For systematization of targeted pieces was used prostate segmentation according to PIRADS (prostate imaging reporting and data system) concept that is adaptation of EAU 2012 card and recommendations of European Society of Urogenital Radiology ESUR based on anatomical and histological prostate structure.

MpMRI was perfomed with the help of device Hitachi Echelon with magnetic field capacity 1,5 T. MRI assessment was made according to international PIRADS system separately for peripheral and central zone. In complex evaluation such MRI parameters as T2, DWI and dynamic contrast enhancement (DCE) were used.

Using T2 regimen we determined heterogenity and local changes defining their size, quantity, zonal localization. Suspicious prostate lesions had depressed signal intensity in T2WI with unequal contour, low water molecule diffusion (high DWI signal). With the help of DCE we determined the quick contrast accumulation and contrast evacuation and subsequent contrast accumulation by surrounding parenchyma.

Findings were estimated according to PIRADS with PC probability, PC aggressiveness, localization and extraprostatic spread. Local changes of prostate tissue were defined and detailed with the need of morphological confirmation. After that MRI data were compared with pathohistological findings of corresponding prostate lesions.

As part of patients had PIN in more than one histological pieces, we analized total amount of lesions that contained PIN. The most often PIN was localized in 1-2 pieces, namely in 21 (41 %) of patients (among them in 1 piece in 7 patients, in 2 pieces in 14 cases). 20 patients (38 %) had PIN in 3-4 specimens (among them in 3 pieces in 11 patients, in 4 pieces in 9 patients). In 11 (21 %) cases PIN was detected in 5 and more specimens (among them in 5 pieces – 6 patients, in 6 pieces – in 3 patients, in 7 pieces – in 2 patients). Total amount of PIN specimens in patients whom was perfomed cognitive fusion MRI biopsy was 166. Among them were 104 specimens of peripheral zone PIN and 62 specimens of central zone PIN.

By evaluation of PIN specimens according to PIRADS criteria were used grades of clinical significant PC risk. 1 grade corresponded to very low (clinically significant cancer is highly unlikely to be present), 2 grade: low (clinically significant cancer is unlikely to be present), 3 grade: intermediate (the presence of clinically significant cancer is equivocal), 4 grade: high (clinically significant cancer is likely to be present), 5 grade: very high (clinically significant cancer is highly likely to be present).

In order to detect PC and to determine prognostic significance of mpMRI rebiopsies with 6 months interval during 3 years were perfomed. MpMRI data of each specimen were compared with histological data of corresponding pieces obtained after rebiopsy.

All described research methods were approved by ethic commission of State institution of science 'Research and practical center of preventive and clinical medicine' State administrative department. All patients gave an informed concent before involving into investigation.

RESULTS

MpMRI data of peripheral prostate zone were studied. Analysis T2WI of 104 PIN specimens established the following results. In 24 (23 %) of PIN cases was observed grade 1 (PIRADS), in 42 (40,4 %) PIN lesions was determined grade 2, in 33(31,7 %) PIN pieces was detected grade 3 and 5 (4,8 %) of PIN specimens had grade 4. In addition, there were no grade 5 data in peripheral zone PIN.

The mean value according to PIRADS in peripheral zone PIN amounted 2,2 (figure 1).

MpMRI data of central prostate zone were studied. Analysis T2WI of 62 PIN specimens established the following results. In 17 (27,4 %) of PIN cases was observed grade 1 (PIRADS), in 26 (42 %) PIN lesions was determined grade 2, in 19 (30,1 %) PIN pieces was detected grade 3. Besides, there were no grade 4 and grade 5 data in central zone PIN.

The mean value according to PIRADS in central zone PIN amounted 2 (figure 2).

Functional parameters of 166 PIN lesions were assessed by studiing of DWI in mpMRI. Analysis DWI of 166 PIN specimens established the following results. In 63 (38 %) of PIN cases was observed grade 1 (PIRADS), in 65 (39 %) PIN lesions was determined grade 2, in 32 (19,3 %) PIN pieces was detected grade 3 and 6 (3,6 %) of PIN specimens had grade 4. In addition, there were no grade 5 data in DWI in patients with PIN. The mean DWI value according to PIRADS amounted 1,7 (figure 3).

Dynamic MRI with contrast enhancement report was negative (no early enhancement, or diffuse enhancement not corresponding to a focal finding on T2W and/or DWI or focal enhancement corresponding to a lesion demonstrating features of benign prostatic hyperplasia on T2WI) or positive (focal, and earlier than or contemporaneously with enhancement of adjacent normal prostatic tissues, and corresponds to suspicious finding on T2 and/or DWI). DCE data were not used in general value in cases of low PC probability (grades 1 and 2) or high PC probability (grades 4 and 5). In cases of PIRADS 3 (in T2WI, DWI) local contrast enhancement in DCE increased the grade to PIRADS 4.

As a result of complex mpMRI (T2WI, DWI, DCE) analysis of all peripheral and central PIN specimens was developed general value according to PIRADS criteria.

The following general values mpMRI among 166 PIN specimens were determined: in 48 (28,9 %) cases was defined grade 1, in 63 (38 %) lesions – grade 2, in 47 (28,3 %) – grade 3, in 8 (4,8 %) specimens – grade 4. There were no grade 5 value among PIN patients determined (fig. 4). The mean PIRADS value in PIN was 2,1.

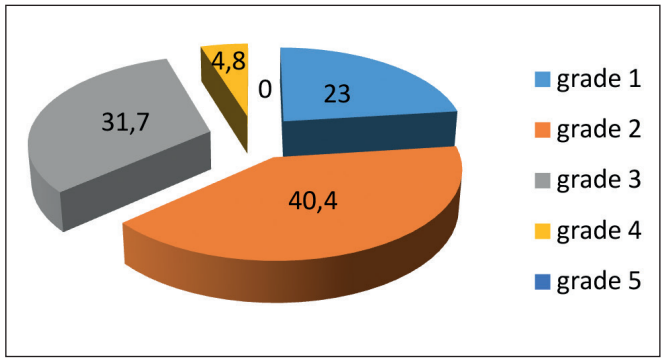

Fig. 1. Structure of T2WI in peripheral zone PIN values according to PIRADS

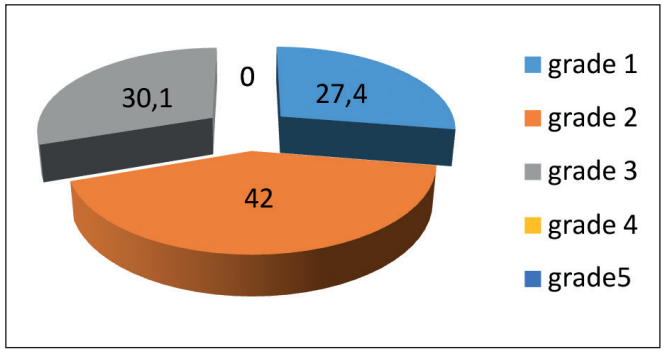

Fig. 2. Structure of T2WI in central zone PIN values according to PIRADS

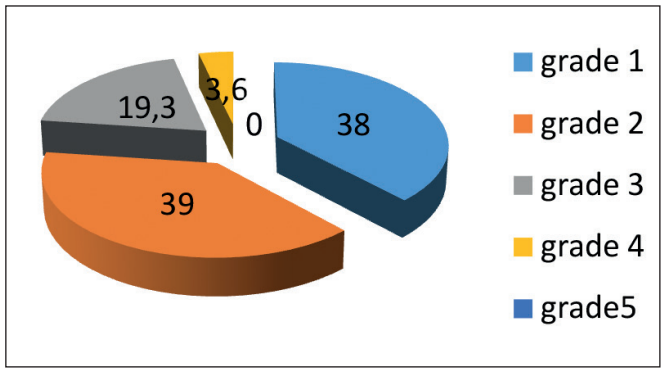

Fig.3. Structure of DWI of PIN lesions according to PIRADS

On the basis of obtained data we came to the conclusion that the situation of mpMRI in patients with PIN is heterogeneous. Though the majority of PIN specimens, namely 67 % has benign tissue attributes (grades 1-2), exists a part of lesions, namely 33 %, which has PC suspicion (grades 3-4). These results had to be detailized to define the group of patients with high risk of malignant transformation of PIN to PC.

During 3-year follow-up with 6 months interval prostate rebiopsies were performed. The specimens with cancer detection in correlation with primary mpMRI data were analized. It was determined that during 3-year follow up of 52 patients who took part in the study PC was diagnosed in 14 men. The total amount of specimens with PC was 45.

The colletion of mpMRI data with corresponding 45 malignant prostate lesions determined the following PIRADS grades: in 5 (11 %) of cases was detected grade 1, in 15 (33,3

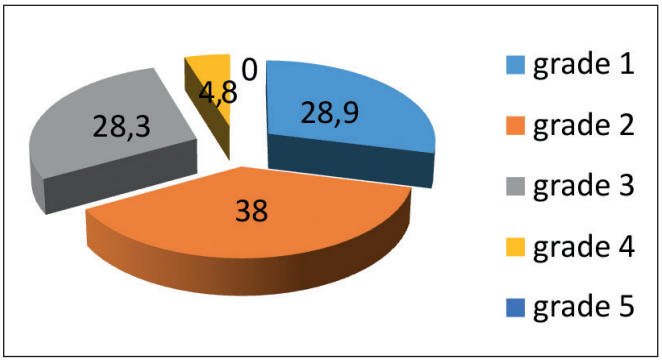

Fig. 4. Structure of PIN lesions according to PIRADS.

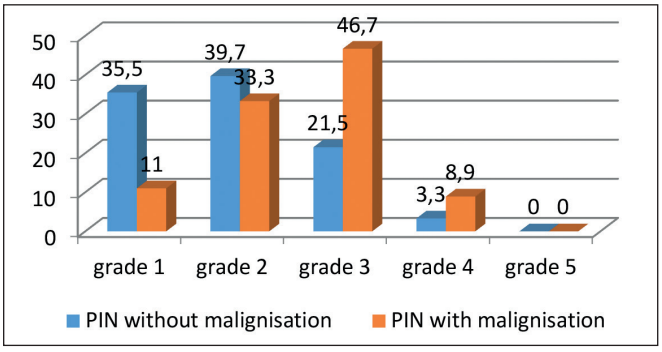

Fig. 5. Distribution of PIRADS grades in PIN specimens corresponding to malignisation

%) lesions was observed grade 2, in 21 (46,7 %) specimens – grade 3, in 4 (8,9 %) was determined grade 4. There were no grade 5 value among PIN patients determined (fig. 5).

The mean PIRADS value of PIN specimens with subsequent malignisation was 2,5 and was in 24 % bigger than in PIN specimens without subsequent malignisation.

DISCUSSION

Obtained results confirm role of PIN in prostate cancerogenesis as transitional state from normal to malignant tissue. Moreover mpMRI data assessed in T2WI, DWI and DCE regimens showed the differences between normal prostate tissue and PIN.

Based on obtained data we came to the conclusion that mpMRI plays an important role not only in PC but also in PIN. Although PIN has no specific MRI signs, PIN specimens are often visualized as suspicious for PC and have common characteristics with adenocarcinoma. Prognostic significance has PIRADS grade, that is complex evaluation of MRI parameters describing morphological picture of prostate lesions. Direct correlation between malignant transformation potential and PIRADS rate was determined.

As a result technical possibilities of MRI contribute to investigation of morphological structure of prostate lesions, namely PIN and PC. Methodology, indications, technical aspects, interpretation of MRI data need futher studiing in order to improve sensitivity and specificity of prostate biopsy.

CONCLUSIONS

According to mpMRI data PIN occupies intermediate place between benign prostate tissue and PC. The mean rate by PIRADS criteria in PIN was 2,1. PIRADS grade has prognostic significance for PIN malignisation. The mean PIRADS grade in PIN specimens with subsequent PC ranged 2,5 and was in 24 % bigger than in PIN specimens without subsequent malignisation. Multiparametric MRI is a sensitive diagnostic method and can be used for detection of patients with high malignisation risk.

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The study was made as a part of research work 'Optimisation of specialized and high specialized surgical treatment based on the 'Fast treck surgery' in thyroid gland, parathyroid gland, nasopharynx, internal and reproductive organs, abdominal wall, blood vessels and joints diseases using atom-power microscopy with application of polarizing mixture for implants processing', which is performed in State institution of science 'Research and practical center of preventive and clinical medicine' State administrative department.

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ORIGINAL ARTICLE

INFLUENCE OF MICROBIOTA ON THE CLINICAL AND IMMUNO-HISTOCHEMICAL CHARACTERISTICS OF CHRONIC GENERALIZED CATARRHAL GINGIVITIS IN CHILDREN

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ABSTRACT

The aim: The purpose of the study is to characterize the influence of quantitative and qualitative composition of gingival microbiota on the status of the main immune system cells, localized in the gums, in chronic generalized catarrhal gingivitis in children.

Materials and methods: The study involved 26 children aged 9 to 16 years, patients with chronic generalized catarrhal gingivitis mild to moderate severity (CGCG) and 18 children with intact gums were comparison group. We determined the hygienic indices Fedorov, has been received, Silness-Loe, PMA, bleeding index for Myuleman and intensity of caries index CFD + cf, CFD. Histological and immunohistochemical studies were performed on serial sections kriostatnyh who made biopsy of gingival papillae. Microbiological study gingival part of crown plaque was performed by multiplexed PCR in real time.

Results: Value hygienic indices in children with CGCG higher than in healthy, indicating the difficulty of care in the presence of periodontal inflammation.

As a result of immunohistochemical studies revealed that HLA-DR + cells under conditions of active disease migrate to mucosal lamina propria epithelium. Number of CD3 + cells in the epithelium CGCG was significantly higher than the number in the intact epithelium and was the most numerous of population. In the biopsy of affected children significantly reduced the number of CD4 + cells. When CGCG quantitative total bacterial mass, *Lactobacillus spp., Enterobacteriaceae, Gardnerella vaginalis / Prevotella bivia / Porphyromonas spp*. in the sample CROWN dental plaque was significantly higher than rates under physiological conditions, and may serve as diagnostic criteria of dysbiosis.

Conclusions: So, CGCG is a disease in the etiology of which is one of the leading roles played by microbial factor, namely, the value of the quantitative ratios of certain types of microorganisms of dental plaque compared to the total bacterial mass of plaque. Therefore, it is reasonable to include comprehensive treatment CGCG drugs in children, leading to natural immunostimulation which causes restoration of local immunity in the gum tissue and drugs to restore quantitative and qualitative composition of normal microflora of the child, thus providing a high therapeutic effect and serve as justification their choice.

KEY WORDS: children, chronic generalized catarrhal gingivitis, samples of gingival tissues, plaque microbial composition

Wiad Lek. 2021;74(1):39-42

INTRODUCTION

Chronic catarrhal gingivitis is currently considered as one of commonly spread oral disease. This disease can result from poor oral hygiene and be regarded as the first stage in the development of periodontitis or periodontal syndrome [1,2]. The risk factors for chronic catarrhal gingivitis typically include poor oral hygiene and some systemic disorders, but changes in the supragingival plaque microbiota structure just above the gingival margin play a leading etiological role [3-5].

The relevance of studies of immune cells, mucosal immunity in chronic catarrhal gingivitis is based on the fact that it is the immune response that is intended to protect, can support inflammation and trigger a cascade of destructive processes towards the body's own tissues [6]. The involvement of the main immune cells of the adaptive immunity in the lesion foci and their role are not completely understood and require detailed investigation of their susceptibility to various factors, and, in particular, their responsiveness to standard and novel therapy methods.

THE AIM

The purpose of the study is to characterize the influence of quantitative and qualitative composition of gingival microbiota on the status of the main immune system cells, localized in the gums, in chronic generalized catarrhal gingivitis in children.

MATERIALS AND METHODS

The clinical study included 26 paediatric patients aged from 9 to 16 years with chronic generalized catarrhal gingivitis of mild and moderate severity according to the classification recommended by the Republican Conference of Dentists of Ukraine (Odessa, 1998), and 18 children in good general health with intact gums, who made up the control group.

All studies were conducted after the signing of the informed consent by patients. The study was approved by the Commission on Ethical Issues and Bioethics of Ukrainian medical stomatological academy.

Clinical dental examination, the establishment of diagnosis of chronic generalized mild or moderate catarrhal gingivitis and its confirmation were performed by using standard techniques [7]. The following clinical indices were assessed: the Fedorov-Volodkina hygienic index (1971), the Silness-Löe plaque index (1967), the PMA, the Muellemann bleeding index, and the caries intensity according to the DEF+df and DEF index.

The first group, a control group (n=18), included individuals with intact gums. Quantitative and qualitative characteristics of gingival microbial plaque were studies in 10 subjects; histological and immunochemical study of gum papillae samples taken from the other 8 individuals were carried out as well.

The second group (n=26) involved patients with chronic generalized catarrhal gingivitis of mild and moderate severity.

Histological and immunohistochemical studies were performed on serial cryostat sections, which were made from 2 mm^3 gum samples obtained under conduction anaesthesia (Sol. Lidocaini 2% 1.5-2.0 ml) from 8 subjects of the control group.

To determine the bacteria present in the supragingival plaque, we studied samples taken from the precervical segment of vestibular surface of two or three front teeth (incisors and canines) close to the gum margin on the upper and / or lower jaws.

RESULTS

Data collected on the oral hygienic status in both the individuals with intact parodontium and in the individuals with chronic generalized catarrhal gingivitis demonstrated its low level. It should be noted that the values of oral hygienic indices in the children with chronic generalized catarrhal gingivitis were higher than in the control that indicates the difficulty in keeping oral care in the presence of parodontium inflammation.

Findings of the dental examination of the children in the first group showed that their gums were pink, moist, shiny, gum papillae were point-ended, densely adhered to the teeth. Caries indexes corresponded to three degrees of the caries intensity.

The results of the periodontal tissue study showed that the PMA index, bleeding index, and Silness-Loe gingivitis index equalled 0 indicating no inflammation in the gums. The absence of chronic inflammation in the gums was confirmed by the negative results of the Pisarev-Schiller test.

The investigation of the morphological structure of the intact papilla showed that the condition of the mucous membrane was in line with the normal values. Immunohistochemical studies revealed that HLA-DR+ cells were located in the spinous layer, where they had numerous processes oriented mainly along the surface of the epithelium. While approaching the basement membrane, the cells lose their processes. These morphological changes reflect the trajectory of the movement of HLA-DR + antigen presenting cells represented by Langerhans cells in the oral mucosa.

An analysis of the CD3 + cells location revealed that their population was not numerous and represented by single cells located within the basal and adjacent spinous layers and in the lamina propria close to the basement membrane. The location and the number of CD4+ cells, in general, were identical to those of CD3+ cells; at the same time CD8+ cells were localized mainly in the projection of the papillae apices in the basal and adjacent spinous layers. CD20+ cells were single and rarely found in the basal and spinous layers, more often in the deep mucosa layers.

Histological examination of the gingival samples of the children with chronic generalized catarrhal gingivitis revealed manifestations of vacuolar epithelium (25% balloonign dystrophy), parakeratosis (88%), edema, and round-cell infiltration of the lamina propria (75%).

Clinical findings in the patients of the second group demonstrated the following: gingival margin and interdental papillae were hyperemic, with cyanotic tinge, swollen, bleeding. The caries indices corresponded to the compensated and subcompensated forms of caries; hygienic condition in 75% of the patients with chronic generalized catarrhal gingivitis was unsatisfactory (2.1 to 5 points), PMA indexes (17% to 50%) and Caries Index (0.3 to 3.0) showed moderate gum inflammation.

Real-time PCR data have revealed that in chronic generalized catarrhal gingivitis, quantitative indicators of total bacterial mass, *Lactobacillus* spp., *Enterobacteriaceae*, *Gardnerella vaginalis* / *Prevotella bivia* / *Porphyromonas* spp. in the samples of supragingival plaque significantly exceed the indicators in health (physiological conditions), therefore they can serve as criteria for the diagnosis of dysbiosis (Table I).

It should be pointed out that in chronic gingivitis the total number of *Lactobacillus* spp. is significantly higher than that in the intact gums.

DISCUSSION

Immunohistochemical study has demonstrated HLA-DR+ cells in the disease actively migrated from the mucous membrane to the epithelium that was confirmed by a significant ($p < 0.05$) increase in their number in the epithelium and a decrease in their lamina propria. The number of CD3 + epithelial cells in chronic generalized catarrhal gingivitis was significantly higher than the number in intact epithelium and constituted the largest share of the population. The count of CD4+ cells in the samples of the second group showed a probable ($p < 0.05$) decrease in the number of these cells in the epithelium and in their lamina propria. Thus, it can be assumed that in chronic generalized catarrhal gingivitis the regulatory potential of CD4+ cells gets impaired. Instead, we observed a redistribution of

Table I. Indicators of dysbiosis in supragingival plaque in chronic generalized catarrhal gingivitis M ± m

Study subjects	Total bacterial mass	*Lactobacillus* spp.	*Entero-bacteriaceae* spp.	*G.vaginalis/ P.bivia / Porphyromonas* spp.
I group	6,38±0,45	5,1±1,4	5,1± 0,44	5,01± 0,68
II group	7,56± 0,3*	6,22 ±0,72*	6,22 ±0,2*	3.50±0,33*

* - reliable difference between groups makes up p<0,05.

CD8+ cells, in which their presence in the lamina propria grew against a moderate decrease in their number in the epithelium. CD8+ cells perform predominantly effector / cytotoxic functions, and their accumulation in the epithelium indicates involvement of these cells in the immune response within the epithelium. The study of the CD20+ cell content revealed their number remained virtually unchanged. Thus, the processes occurring in the tissues of the gums in chronic generalized catarrhal gingivitis are consistent with the immune response of the cytotoxic type that undoubtedly indicates the necessity to use agents directed at eliminating the microbial factor [6].

The clinical significance of dysbiosis in the gingival plaque of consisted in denoting the correlation between clinical indices for chronic generalized catarrhal gingivitis and quantitative microbiota ratios. Thus, the results of statistical analysis using the Spearman nonparametric correlation test showed that quantitative indicators of *Lactobacillus* spp. and *Candida* spp. were negatively correlated ($p < 0.05$) with the Fedorov-Volodkina hygiene index ($R = -0.85$; $R = -0.66$, respectively) in chronic generalized catarrhal gingivitis [3].

In chronic generalized catarrhal gingivitis, we have found out significant correlations ($p < 0.05$) between the Silness-Loe hygiene index and the *Eubacteridacea* spp., ($R = 0.60$), and the negative correlation between the bleeding index and *Lactobacillus* spp. ($R = -0, 85$). According to the relevant report [3,8], this may reflect the value of *Eubacteridacea* spp. as an indicator of an increase in the total plague amount, and the predominance of autochthonous commensal species among Lactobacillus spp.

CONCLUSIONS

Thus, chronic generalized catarrhal gingivitis can be regarded as a disease, in aetiology of which a microbial factor and, namely, the value of the quantitative ratio of certain types of microorganisms in the supragingival plaque compared with the total bacterial mass of dental plaque plays a leading role. Accordingly, the action of microbial pathogens disrupts the barrier function of the epithelium and induces immunological responses in the mucous membrane, and the main effector processes take place within the epithelium, involving HLA-DR +, CD3 +, CD4 +, CD8 + cells.

It is these characteristics that determine the reversibility of these pathological changes when using etiotropic therapy (antiseptic agents). However, using exclusively antiseptics leads to a sharp decrease in the degree of antigenic immunostimulation and microbiological parameters. Therefore, it seems to be appropriate to include medications resulting in the natural immunostimulation, which, in turn, promotes the local immunity recovery in gingival tissues, as well as medication restoring quantitative and qualitative microbiota structure, into the integrated therapy of chronic generalized catarrhal gingivitis in children.

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ORIGINAL ARTICLE

THE EFFECT OF DIET ENRICHED WITH PYROPHOSPHATE (E450) ON EXPRESSION OF GENES ENCODING BONE MORPHOGENETIC PROTEIN AND OSTEOCALCIN IN MOUSE EMBRYONIC MANDIBLE TISSUES

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ABSTRACT

The aim: Of our study was to measure the mRNA expression of the investigated odontogenesis factors in mandible tissue of mouse embryos (17th day of pregnancy) gestated by females, kept on a E450 rich diet since 30 days before fertilization to gestation.

Materials and methods: The effect of food supplements was studied in «Overload phosphates model». Experiments were carried out on white nonlinear outbred mice with mass 25–28g (n=40). The females from the control group were fed with standard rodent food, whereas the experimental females were fed with pyrophosphate-enriched food. The materials, used for the molecular genetic study, were the lower jaws of 17-days old mouse embryos (*E–17*).

Results: The investigated *BMP2* and osteocalcin genes are expressed at approximately the same level. Pyrophosphate-rich diet does not alter *BMP2* gene expression, but it significantly increases the expression of *osteocalcin*.

Conclusions: The present study is the first one to describe the impact of the pyrophosphate-rich diet on mRNA expression of key osteogenesis regulators – *osteocalcin* and *BMP2*.

KEY WORDS: Bone morphogenetic protein 2 (*BMP2*) expression, *osteocalcin* expression, lower jaws of mouse embryos, pyrophosphate-rich diet, food additive E450

Wiad Lek. 2021;74(1):43-47

INTRODUCTION

An optimized and well-balanced prenatal diet is essential for the physiological fetal development and formation of oral cavity organs and tissues in embryos [1, 2]. Negative effects of food preservatives and colorings on human health, especially during pregnancy, are among greatest health concerns of our time [3, 4].

The World Health Organization (WHO) defines food additives as chemical substances and natural compounds, which are added to alimentary products for improvement in raw materials and end product quality. In Ukraine, the following definition (which does not go beyond the WHO one) is adopted: food additives are natural or artificial substances and their compounds, which are added to alimentary products at the production stage to give them specific properties and (or) preserve their quality.

Food additives are substances added to alimentary products due to the processing reasons, namely for preservation of organoleptical properties and extension of shelf life.

The food additives consumption by the population has significantly increased in recent years. This is due primarily to the expansion of the list of substances authorized for addition to food. A big deal of daily use products contains some amount of food additives (cereal and meat products, cheeses, oils, mayonnaise, NA beverages, confectionary goods). The world production of food additives is continuously increasing, both quantitatively and qualitatively: by 10-15% in Asia, by 4,4% in the USA, and only by 2% in Europe [5].

We focused our attention on one of the stabilizers, encoded E450, which is a pyrophosphate $H_4P_2O_7$ and it is authorized as a stabilizing agent, though in some countries it is classified as hazardous to health [6]. It can be found in meat products, sausages, bacons, semi-finished products, jam, condensed milk, chocolate and processed cheese spreads, lemonade, sugary foods, etc [4]. Despite this, E450 is classified as a food additive, causing gastrointestinal and kidney diseases and calcium-phosphorus imbalance with further development of osteoporosis. It should be noted, however, that pyrophosphates are safe if consumed moderately [4].

Excessive intake of phosphates impairs calcium absorption [5], what can be crucial at the tooth bud mineralization stage [7]. The impact of maternal nutrition on the fetal odontogenesis has been well studied, but there are no studies dealing with teeth germination disorders, caused

by excessive pyrophosphate (food additive E450) intake.

Of special interest are the studies, focusing on expression of the genes, the protein products of which play a crucial role in the mentioned processes at all stages of odontogenesis. Considerable attention is being paid to the bone morphogenetic protein (encoded by *BMP2* gene) and the *osteocalcin* (*Bglap* gene) because of their dominant role in the calcification of a tooth bud [8, 9, 10].

It was investigated, that the *BMP2*, which is a growth factor for the tooth bud cells [11], promotes the differentiation of follicular cells into cementoblasts / -osteoblasts [12]. Such *BMP2* action is caused by the expression of a number of genes (type I collagen, osteonectin, dentin sialophosphoprotein, nestin) in cells of dental pulp [13]. The expression of *BMP2* gene, in turn, is controlled by the growth hormone (somatotropin) and insulin-like growth factor-1, that both can increase the *BMP2* expression in dental pulp fibroblasts *in vitro* in 4–5 times [14]. Moreover, the differentiation of enamel organ cells is also regulated by growth factors, in particular by transforming growth factor alpha (TGF- α) and epidermal growth factor (EGF) [15]. The *BMP2* protein is able to activate the receptors of osteoblasts located on the tooth germ surface and stimulate the proliferation of dental pulp mesenchymal cells with following differentiation in odontoblasts, thus providing the formation of osteodentine and tubular dentine [14]. *BMP2* is predominantly expressed in epithelial cells at the early stage (up to 13th day of a mouse embryo development) of tooth development. However, at the later stages it begins to be expressed mainly in the mesenchymal cells of dental papilla, initiating the intensification of dentinogenesis. Thus, *BMP2* is responsible for dental mesenchyme cells' fate during the tooth formation [16].

Odontoblasts also synthesize calcium-binding proteins - osteocalcin and osteonectin, which are expressed in dentine as well as in bone tissue [15]. Osteocalcin belongs to the proteins containing three residues of γ-carboxyglutamic acid that binds free calcium and prevents the apatite formation. Osteocalcin is a vitamin K-dependent matrix protein, which is able to bind to hydroxyapatites. It is synthesized by the dentine odontoblasts and it is a key factor in dental connective tissue mineralization [17].

We failed to find any published data regarding the effect of excessive pyrophosphates intake by a mouse female during pregnancy on mRNA expression of *BMP2* and *osteocalcin*.

THE AIM

The aim of this study was to measure mRNA expression of these odontogenesis factors in mandible tissue of embryos (17th day of pregnancy) gestated by females, kept on a E450 rich diet since 30 days before fertilization to gestation.

MATERIALS AND METHODS

In calculating the diet for the experimental mice group we have considered such data as: the actual nutrition of pregnant women, which was evaluated by questionnaire-polling method [1], recommended daily set of products for a pregnant woman[1], the content of E450 in foodstuffs, M.A.C.[2] of E450 in some foodstuffs[3]. Approximate daily set of products for a pregnant woman: 200g of meat or fish, 1 liter of milk in any form, 100-150g of curd, 20-30g of cheese, 1 egg, 600g of vegetables, 200-300g of fruits.

The effect of this food additive was studied in «phosphate overload model» (we add 2 g of E450 (sodium pyrophosphate, Israel) per 100g of standard rodent food). We did not apply for any modification to a basic model [18].

Experiments were carried out on white nonlinear outbred mice with mass 25–28g (n=40). All mice were separated into 2 groups: control group and experimental group. The females from the control group were fed with standard rodent food, whereas the experimental females were fed with pyrophosphate-enriched food (2% of pyrophosphate, Israel). Females in proestrus or estrus phase were kept with the males in proportion 4:1 30 days later. The presence of spermatozoa in the vaginal smear was considered as an indicator of fertilization and first day of pregnancy. Animals were fed with standard food (control group) or pyrophosphate rich food (experimental group). Pregnant mice (n=6 per group) were sacrificed by the carbon dioxide expose on the 17th day of pregnancy (E–17). Mousekins were sacrificed on day 2 (*D–2*) or day 28 (D–28) after the birth. Experiments were performed in accordance with the European Community Standards.

The material of the molecular-genetic study were the lower jaws of 17-days old mouse embryos (*E–17*), since *bone morphogenetic protein 2* (*BMP–2*) and *osteocalcin* (encoded by *Bglap*) [8, 9, 10] are crucial for the tooth bud calcification at all stages of odontogenesis, especially at the bell stage (period from 16.5 to 18.5 days of pregnancy) [19].

RNA samples were extracted from mandible tissue with phenol/chloroform using *Sigma-Aldrich* (USA) reagents. RNA concentration was measured by NanoDrop 1000 spectrophotometer by *Thermo Scientific* (USA). Reverse transcription was performed on 200-300 μg of total RNA using *First Strand cDNA Synthesis Kit* (*Fermentas,* Lithuania) and $(Oligo(dT)_{18}$ Primer. The resulting cDNA product was used for PCR amplification. Quantification of BMP2 and *bone gamma carboxyglutamate protein* (*Bglap, osteocalcin*) expression was performed by real-time PCR using primers shown below:

BMP2 Up: 5`-GTGGAGGAACTTCCAGAGATGA-3`;
BMP2 Dw: 5`-CTGCAGATGTGAGAAACTCGTC-3`;
Osteocalcin Up: 5`-CAGGAGGGCAATAAGGTAGTGA-3`;
Osteocalcin Dw: 5`-CAGGGTTAAGCTCACACTGCTC-3`.

[1]Maximum allowable concentration.
[2]Maximum allowable concentration of the food additive E450 in some foodstuffs: baked goods - 10 000 mg / kg, desserts - 3000 mg / kg, ice cream - 1 000 mg / kg, flour - 2 500 mg / kg, raw eggs - 10 000 mg / kg, sauces - 5000 mg / kg, processed cheese - 9000 mg / kg, meat and fish products - from 100 to 5000 mg / kg (0.3% of the total weight of the forcemeat).
[3]The maximum acceptable daily consumption of E450 is 70 mg/kg body weight.

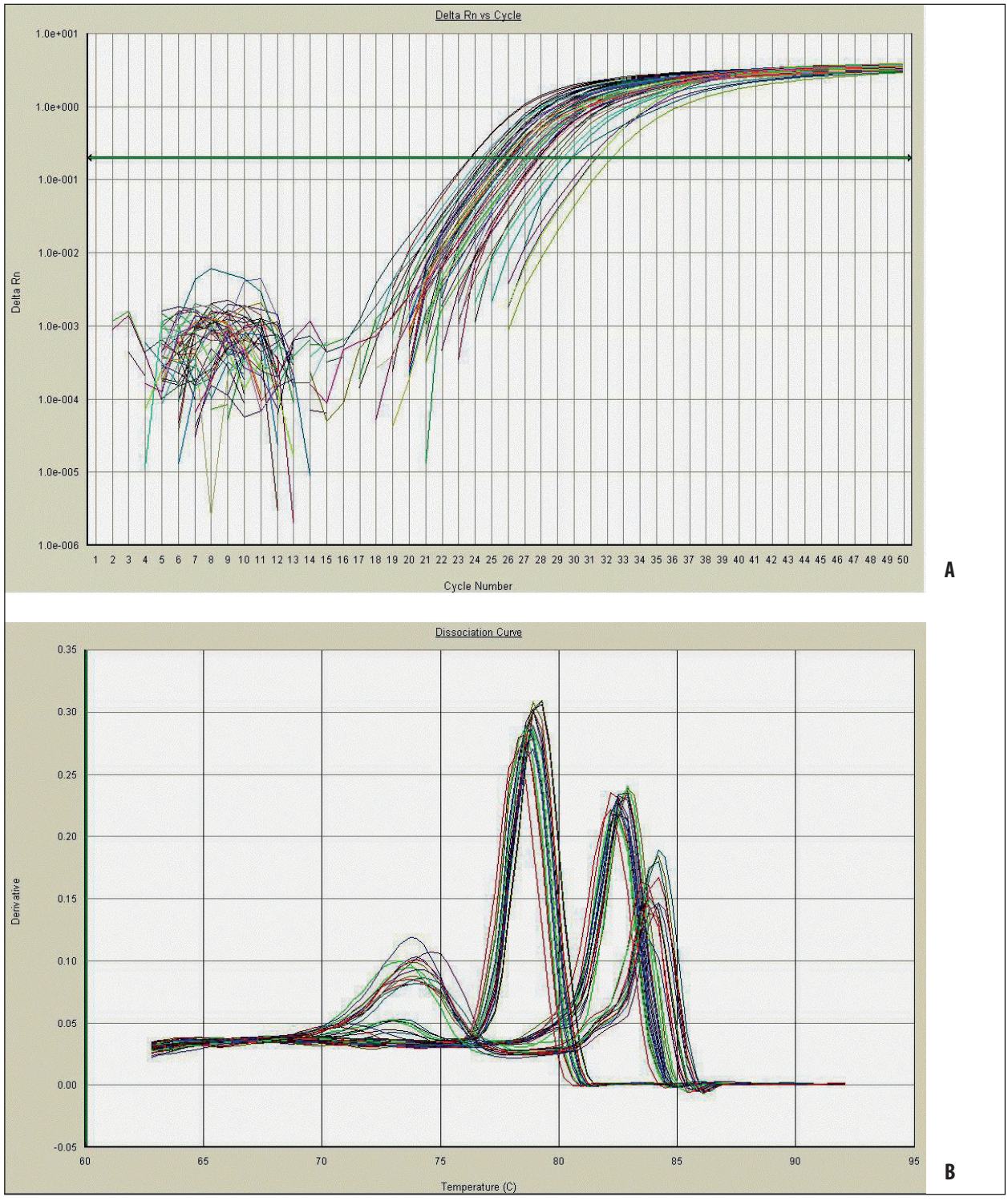

Fig. 1. Actin, BMP2 and osteocalcin amplification products concentration relative to amplification cycle number curve (A) and amplification products dissociation curve (B).

PCR amplification reactions were performed in 20 μL of SYBR Green *PCR Master Mix* containing 25 pM of each primer. Thermal cycling conditions comprised an initial denaturation and AmpliTaq Gold® DNA polymerase activation step at 95°C for 10 min, followed by 50 cycles of denaturation for 15 s at 95°C and 60°C (or 61°C, in case of *osteocalcin* gene) for 1 min. In addition, melting curve analysis was performed to control the specificity of PCR product fluorescence: the sample was *gradually* heated from 60(61) to 94° C while simultaneously detecting decrease of DNA-*SYBR Green* complex fluorescence intensity.

Amplification products concentration vs cycle curve and amplification products dissociation curve are shown in fig. 1.

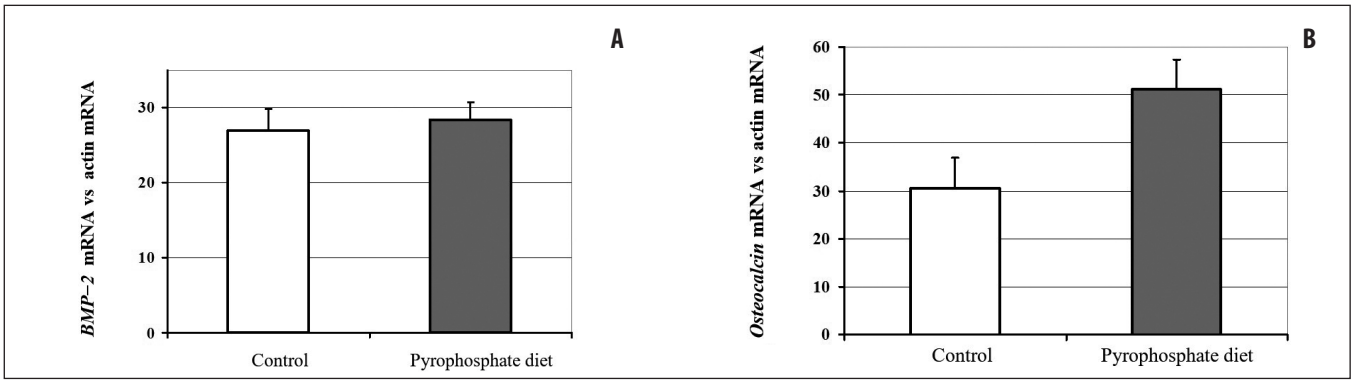

Fig. 2. BMP2 (A) and osteocalcin (B) mRNA expression relative to actin in lower jaw tissue of mouse embryos from control and experimental (high pyrophosphate diet) groups.

The analysis of the data received was performed using 7500 Fast Real-time PCR Software.

The extrapolation of the obtained results to humans is theoretically possible due to the great described genes sequence similarity between humans and mice [20, 21].

Statistical analysis. Statistical analysis of the digital data was performed using Excel 2000 and Origin 7.0. Probability distribution of mean ($P<0.05$) was calculated using Student's t-test.

RESULTS AND DISCUSSION

The expression of *BMP2* in jaws was the same in animals from control and experimental groups ($p=0,71$; $p>0,05$). Nevertheless, pyrophosphate-enriched diet caused 1,8-fold increase of *osteocalcin* expression ($p=0,047$; $p<0,05$) to 51,2±6,20 (fig. 2).

The analysis of the data shows that a diet with high content of sodium pyrophosphate does not change the expression of *BMP2* gene. Given that *BMP2* is a key factor of odontoblasts differentiation [22], we can hypothesize that excessive pyrophosphate in maternal diet would not influence the odontogenesis in the embryo. However, sodium pyrophosphate rich diet is likely to increase the expression of osteocalcin. On the one hand, raised osteocalcin expression seems to be a positive sign, since osteocalcin ensures the mineralization of tooth bud tissues, and that means intensification of apatite formation in animals with gained expression of osteocalcin. But on the other hand, the hyperexpression of osteocalcin could cause the premature tooth mineralization that can disrupt the processes of teeth formation and odontogenesis all in all.

Our results do not fully agree with those in the study [23], where the authors researched the effect of inorganic phosphate/pyrophosphate on expression of a wide range of genes (using real-time PCR) in mouse cementoblasts. It was investigated that pyrophosphate at the concentration of 5mM caused increase of ogosteopontin and dentin matrix protein-1 expression and decreased mRNA expression of bone sialoprotein (Bsp), osteocalcin and type I collagen. Differences in the results could be explained by the differences in selected methodology (experiments were conducted in vitro on isolated cells) and by briefness of pyrophosphate exposure (to 48 hours). Our study shows the effect of 50 days-long pyrophosphate exposure of the whole organism. We evaluated the gene expression in lower jaw tissues instead of using cultivated cells for this purpose. Nevertheless, the most important fact is that pyrophosphate can alter the expression of some genes significant for odontogenesis.

CONCLUSIONS

Thus, we first described the impact of pyrophosphate-enriched diet on mRNA expression of *osteocalcin* and *BMP2* which are crucial regulators of osteogenesis. Further observations on histopathological changes in mouse tooth bud would give a possibility to match the genetic changes with the pathomorphological changes and clarify the functional role of the alterations in expression of the investigated genes.

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

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

ORIGINAL ARTICLE

TELEREHABILITATION OF THE KNEE JOINTS OF PATIENTS WITH POLYTRAUMA

10.36740/WLek202101109

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ABSTRACT

The aim: The overarching theme of this paper is to discuss implementation of the telemedicine technology for rehabilitation of patients with injuries of the lower extremities.

Materials and methods: Consecutive patients were recruited over a four-year period. A total of 48 polytrauma patients with lower extremity injuries were enrolled in the study after the resolution of the surgical sequelae. 16 patients from the control group underwent traditional rehabilitation procedures for a 3-week period after injury. A total of 32 subjects were enrolled in the telerehabilitation group for a 3-week study period after injury and were trained with a set of exercises for home use. Home remote monitoring for the 96 test subjects included use of a Portable device with Axis-sensor, temperature and volume sensors, that were fixed to the injured limb. Software permits the monitoring of exercise time, local temperature, the biomechanics of active movements of the injured limb.

Results: The orthopedic surgeon during telerehabilitation took significantly less time to consult patients (1.9 minutes) than the traditional rehabilitation (15.2 minutes). Patient satisfaction was higher for the telerehabilitation with machine learning algorithm (78.3%) than for the orthopedic surgeon's traditional rehabilitation (36.7%).

Conclusions: Subjects reported a higher satisfaction with telerehabilitation than with the traditional orthopedic rehabilitation due to the fact that they spent less time at the hospital and had more time for exercises at home under orthopedic remote monitoring. The telerehabilitation system can be used in complex rehabilitation of patients with injuries of the lower extremities.

KEY WORDS: telemedicine, rehabilitation, polytrauma

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INTRODUCTION

The continuous development of technology that paves the way towards the expansion of connections through the internet and the growth of the capacity to process data has created greater possibilities of the development of the global health industry especially telemedicine [1]. Telemedicine is the application of transferring medical information in through digital communication to perform consultations, medical examinations and rehabilitation procedures. The main objectives of telemedicine are bridging the gap of accessibility and communication in the medical field reducing delays and the cost logistics [2]. Wireless technology applied to sensors and application to case studies related to home monitoring and have been developing during the past decade, including studies on the cost-effectiveness [3]. Polytrauma patients have numerous risk factors. Early assessment of the clinical status of patients with polytrauma is of pivotal importance in guiding surgical and future rehabilitation [4].

The use of a device such that a person may wear, may allow for constant monitoring of a patient and for the ability to notice changes that may be less distinguishable by humans [5].

Timely access to orthopedic rehabilitation is an important problem for the world health care system. Presently, wait times to see an orthopedic surgeon can exceed two months and with the rapidly aging population as well as the increased incidence of obesity, the need for orthopedic care is expected to drastically increase in the coming years. Efforts have been made to ensure timely access to orthopedic care for the population yet, despite an investment in resources, wait times for a consult remain excessively long [6].

Therefore, the overarching theme of this paper is to acknowledge the gold standard in implementation of the telemedicine technology for rehabilitation of the patients with injuries of the lower extremities. The main prerequisite for success within the orthopedic community is to identify indications and contraindications for distance rehabilitation [7]. Lower Extremity Functional Scale is widely used to evaluate the functional disabilities of a patient with a lower extremity disorder. The LEFS score ranges from 0 to 80 with a higher score indicating a higher functional status. The use of the LEFS in research studies has been validated and the LEFS is a reliable tool for assessing lower extremity functional status [8].

An application telemedicine for monitoring and gathering data on a patient's progress without the use of video communication or consultation is the study on joints Rehabilitation System with Feedback from the Wireless Prototype [9-11].

Fig. 1. Portable device with sensors.

Joints rehabilitation system connected through internet to record the number of use and effects of telerehabilitation therapy and using measure the efficacy of the rehabilitation strategy. This is an example of the importance of wireless vital sensors to telemedicine with its capacity to easily transmit data to devices which is vital in continuous monitoring. Through the current developments in patient monitoring, there is a support for self-diagnosing techniques and application of telemedicine not only in hospitals but also in homes [12]. As the capacity expands with telemedicine being applied on devices outside the hospitals, each device should be designed with a standard data architecture.

THE AIM

The overarching theme of this paper is to discuss implementation of the telemedicine technology for rehabilitation of patients with injuries of the lower extremities.

MATERIALS AND METHODS

Consecutive polytrauma patients were recruited after recovery over a five-year period - September 2018 to February 2020 - from the waiting lists of the department of orthopedic surgery, at Ternopil Emergency Hospital that serves as a trauma center. A total of 48 polytrauma patients with lower extremity injuries were enrolled in the study after the resolution of the surgical sequelae and monitored during a 3-weeks period.

All enrolled subjects signed a consent form prior to participating in the study. The Research Ethics Board of the I. Horbachevskyy Ternopil Medical University, Ukraine approved the research study.

16 patients from the control group underwent traditional rehabilitation procedures for a 3-weeks period after injury. This usual care group included the generally accepted methods of rehabilitation therapy (i.e. massage, myostimulation, walking without a load on the injured limb, and exercises in the pool).

A total of 32 subjects were enrolled in the telerehabilitation group for a 3-week study period after injury and were trained with a set of exercises for home use.

Home remote monitoring for the 32 test subjects included use of a Portable device (Fig 1.) with Axis-sensor, temperature, volume and pulse sensors, that were fixed to the injured limb. Software permits the monitoring of biomechanical movement time, local temperature, the frequency of active movements and volume of the injured limb. During the execution of home exercises, data from the subjects Portable device were measured and sent to a server through a cellular Internet connection and to the personal smartphone of the rehabilitation doctor and displayed as digital data and graphically.

The Tele-rehabilitation protocol included:

- Fixation of the Portable device to the injured limb and use of customized software.
- Home exercises: Passive flexion-extension in the leg.
- Home exercises: Active flexion-extension in the leg.

Patients were also trained for subjective assessment of pain on a 10-point scale. The initial implementation of this telerehabilitation model did not include prescription of medications or joint injections.

The software contained a tailored personal rehabilitation record for health information and communication between the patient and health professionals. Basic measures for patients included blood pressure, volume of limb, waist, chest, pulse, weight, height, sex, saturation, local temperature, movement activity and condition of connective tissue.

Subjects completed a questionnaire where they provided anthropometric data as well as information on their education, employment, household income, household living status, and information on clinical variables such as the joints effected, the reason for consulting, the duration of their symptoms, the use of a walking aid and the presence of any co-morbidities. All subjects from both groups also completed the Lower Extremity Functional Scale (LEFS) questionnaire. The majority of patients (75%) cited pain as the reason for consult with the orthopedic surgeon and all of the patients (100%) consulted for a knee disorder (after injuries of the lower extremities). All patients had imaging tests available in their file at the time of consult (X-Ray and CT-scan).

Based on the patient's individual condition, the rehabilitation doctor created an individualized rehabilitation plan for each subject, containing an activity plan. All patients had personal goals for daily movement activity and steps in the rehabilitation program.

All subjects from telerehabilitation group were assigned a personal doctor. The control group received traditional rehabilitation at home without a personal doctor.

RESULTS AND DISCUSSION

In the telerehabilitation arm of the study, 48 subjects with injuries of the lower extremities were symptomatic for at least 3 weeks.

Subjects from telerehabilitation group had a mean age of 44.7 years and the majority were men (67.3%). The mean

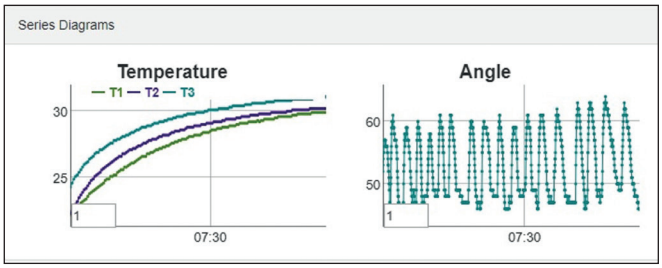

Fig. 2. First day of telerehabilitation.

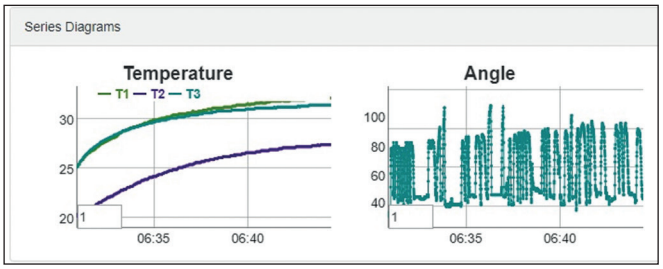

Fig. 3. 14th day of telerehabilitation.

Table 1. Characteristics of the study participants of the telerehabilitation group (n=32)

Characteristics	n (%)	Mean (SD)
Age (years)		44.7 (5.4)
Gender		
Male	21 (65.6 %)	
Female	27 (34.4 %)	
Body mass index (kg/m^2)		27.6 (3.6)
Employment		
Employed	17 (35.4 %)	
Unemployed	15 (31.2 %)	
Retired	-	
Duration of symptoms (days)		14 (3)
Patients using walking aid	39 (81.3 %)	
Lower Extremity Functional Scale – LEFS score (%)		44.61 (6.68)

Lower Extremity Functional Scale (LEFS) score of patients was 44.62. Table 1 presents selected characteristics of the participants of the telerehabilitation group.

In the control group of the study, 16 subjects with injuries of the lower extremities were symptomatic for at least 3 weeks and continued rehabilitation at home without an assigned doctor. Subjects had a mean age of 48.6 years and the majority were men (65.4%). The mean Lower Extremity Functional Scale (LEFS) score of patients was 38.31.

During the telemonitoring, the physician controls the adequacy of execution of each stage of rehabilitation exercises and has the ability to adjust the load in real time depending on the functional state of the limb (Fig 2, Fig. 3.).

Subjects were also asked if their pain level increased after the first exercise and in the event that it did, they were asked to indicate by how much it increased by picking one of the following three options on the smartphone: 1-4 pain was a bit stronger; 5-7- pain was moderately stronger; 8-10 pain was much stronger. Software allows increasing the daily load, if the assessment of pain after exercise was not more than 7 points on 10-point scale and progressive limb edema was absent. If pain persisted or questions persisted, there was correction of the rehabilitation algorithm by doctor.

The orthopedic surgeon during telerehabilitation took significantly less time to consult patients (1.9 minutes, SD:0.5) than the traditional rehabilitation (15.2 minutes, SD:2.7). Patient satisfaction was higher for the telerehabilitation (78.3%, SD:12.6) than for the orthopedic surgeon's traditional rehabilitation (36.7%, SD:7.3) (Table 2).

Subjects reported a higher satisfaction with telerehabilitation than with the traditional orthopedic rehabilitation due to the fact that they spent less time at the hospital and had more time for exercises at home under orthopedic remote monitoring. It is assumed that less clinical time per subject using telerehabilitation translates into reduced costs of rehabilitation during the study period.

The telerehabilitation system can be used in complex rehabilitation of patients with injuries of the lower extremities. This will improve the quality of life in this group of patients and significantly reduce the cost of the rehabilitation period. These results provide preliminary evidence supporting the telerehabilitation model for orthopedic care. We conclude that telerehabilitation should be considered a key component in the long-term management of patients who have lower extremities injuries.

We are at the age of development of telemedicine, a technology that can exceed the capabilities of and can greatly enhance manual procedures and even existing technology. The simplicity of being able to apply this technology allowed it to be of great use in telerehabilitation and this paper shows importance of its implementation to the field of medicine.

CONCLUSIONS

An application of telemedicine is versatile process and it has endless possibilities of development, an application can mean accommodating more patients or discovering the best practice for a telerehabilitation, which will improve quality of patient's life. The implementation of these researches will be the most important contribution, which

Table 2. Comparison between visit time length and patient satisfaction for telerehabilitation (n=32) and traditional orthopedic rehabilitation (n=16)

	Mean value for telerehabilitation (SD)		Mean value for traditional rehabilitation (SD)	
Visit time length, in minutes	1.9	0.5	15.2	2.7
Patient satisfaction, %	78.3	12.6	36.7	7.3

is why it is also important to begin researching on how this technology can be made more cost effective so it can be used in rural areas and underdeveloped hospital facilities.

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ORIGINAL ARTICLE

THE EFFECT OF SMOKING ON NUTRITIONAL STATUS, SEVERITY OF THE DISEASE AND THE DEVELOPMENT OF SYSTEMIC EFFECTS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

The aim: To determine the effect of smoking on indicators of nutritional status in patients with chronic obstructive pulmonary disease (COPD).

Materials and methods: A study included 91 patients with COPD. Patients were divided into two groups depending on the status of smoking: smokers and non-smokers. Everyone underwent an assessment of the severity of COPD, a study of nutritional status, a laboratory study of kidney function and blood lipid profile.

Results: It was found that in smokers with COPD, the disease proceeds with more pronounced shortness of breath. There is also a development of sarcopenic obesity in those patients who smoke. In turn, it was found that bronchial obstruction increases with a decrease in muscle tissue content. Renal filtration function is reduced in smokers and non-smokers.

Conclusions: 1. Patients suffering from COPD have a violation of nutritional status. Smoking patients develop sarcopenic obesity, which progresses with an increase in the degree of nicotine addiction, correlates with the "pack / year" index and is a predictor of increased mortality in this category of patients. 2. Increased bronchial obstruction in smokers with COPD is observed with an increase in smoking history, the number of cigarettes smoked and with a decrease in body weight. 3. Reducing the pool of muscle tissue can be considered as an early predictor of more frequent exacerbations in smoking patients with COPD. 4. The systemic effects of COPD include impaired renal function, more pronounced in smokers with COPD.

KEY WORDS: COPD, nutritional status, smoking, renal function

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an urgent problem of modern pulmonology [1,2,3]. A feature of the disease is not only local damage to the respiratory tract, but also the development of systemic manifestations [4,5,3]. The main etiological factors for the development of COPD include tobacco smoking, air pollution in the industrial region, dust in the workplace and other factors. Data from large-scale population-based studies in recent years have shown that patients with COPD who do not smoke have a minimum total death rate with standardization by age with a body mass index (BMI) of 22.5–24.9 and 20–25 kg / m2 [6]. Patients with more severe bronchial obstruction and BMI <25 kg / m2 have a higher risk of death compared with patients with COPD who are overweight and even obese [7]. It can be assumed that with an increase in BMI in patients with COPD, a more favorable outcome occurs. This phenomenon is also called the obesity paradox in COPD and it can be associated with the influence of adipose tissue on the respiratory mechanism (for example, the relative decrease in static pulmonary volumes in patients with COPD and obesity) [8,9]. A relationship was also established between a decrease in muscle tissue content in patients with COPD and an increase in the severity of the condition of these patients [10]. It is known that adipose tissue can be redistributed from subcutaneous fat to visceral, which is characterized by an increase in cardiovascular risk in patients with moderate COPD [11]. However, perhaps there are other mechanisms of the disease that are interrelated with the risk of mortality and the content of adipose tissue [12].

THE AIM

To determine the effect of smoking on indicators of nutritional status in patients with COPD, to identify the relationship between muscle and adipose tissue levels, nicotine dependence, smoking duration, "pack / year" index, severity of the disease, and the development of systemic effects in patients with COPD.

MATERIALS AND METHODS

A prospective study included 91 stable outpatients with COPD of clinical groups B and C according to the GOLD. The diagnosis of COPD was established according to the

Table I. Clinical characteristics of control groups.

Characteristics	Group I (non-smokers)	Group II (smokers)	p
Age, y M (SD)	55,8 (6,7)	58,3 (8,1)	0,1
Dyspnea severity mMRS, M (SD)	2(1;3)	3(2;4)	0,047
Exacerbation rate (SD)	1(1;2)	1(1,3)	0,6

Table II. Spirometry indices in the examined patients.

Characteristics	Group I (non-smokers)	Group II (smokers)	p
FVC, % Me [25 %-75 %]	85,0 (77,0-92,0)	87,5(70,0-96,0)	0,8
FEV1, % Me [25 %-75 %]	51,0 (44,0 -62,0)	45,0 (34,0 -59,0)	0,04
FEV1%M Me [25 %-75 %]	54,5 (37,0-65,0)	48,0(54,0- 62,0)	0,3
MEF 75, % Me [25 %-75 %]	53,0 (41,6-68,6)	43,0 (31,3-56,4)	0,6
MEF 50, % Me [25 %-75 %]	19,5 (25,5-51,5)	40,0 (18,7-55,2)	0,2
MEF 25, % Me [25 %-75 %]	22,0 (23,8-48,0)	31,0 (15,4-34,7)	0,6
PEF, % Me [25 %-75 %]	54,5 (37,0-65,0)	50,5 (30,7,0-65,0)	0,5
IC_F, % Me [25 %-75 %]	57,5 (47,2-69,6)	52,5 (17,7-65,0)	0,8

recommendations of GOLD 2018 (2). Patients were treated during 2018-2019 years.

Criteria for inclusion in the study: verified diagnosis of COPD in clinical groups B and C, stable phase. The exclusion criteria from the study were: patients over 80 years old, history of acute cardiovascular events, clinically significant heart rhythm disturbances, previously diagnosed diabetes mellitus, kidney diseases, cancer, surgical operations during the last year.

The patients underwent a general clinical examination, including the assessment of complaints, medical history, determination of anthropometric indicators (height, weight, BMI, waist circumference), physical examination. The severity of COPD was determined on the basis of the frequency of exacerbations during the year, assessing dyspnea using the mMRS scale (The Modified Medical Research Counsil Dyspnea Scale), spirographic data (forced expiratory volume in 1 second (FEV1), and forced lung capacity (FVC), ratio - FEV1% M). Additionally, the status of smoking and nicotine addiction (Fagerstrom Test for Nicotine Dependence - FTND) was evaluated. The percentages of total fat, muscle mass and visceral fat were determined on the basis of bioimpedancemetry data (Omron analyzer). Data were compared with reference values (13). The state of lipid metabolism was studied by the enzymatic method on a Humalayzer biochemical analyzer. The levels of total cholesterol (H), low density lipoprotein cholesterol (LDL cholesterol), high density lipoprotein cholesterol (HDL cholesterol) and triglycerides (TG) were determined. We also performed a quantitative analysis of C-reactive protein (CRP), investigated serum creatinine level, urine creatinine level, and glomerular filtration rate (GFR) was calculated using the CKD-EPI formula (Chronic Kidney Desease Epidemiology Collaboration), currently recommended for application as the most suitable in outpatient and clinical practice screening method for assessing glomerular filtration rate. The CKD-EPI method is considered universal and accurate at any stage of CKD [14]. All results obtained were compared with reference values.

The data obtained were processed using the program Statistica 10.0. with the determination of mean values (M), standard deviation (SD) in the case of a normal distribution of values, and the median (Me), upper and lower quartiles ([25%; 75%]) in the case of a non-normal distribution. Parametric methods were used using Student's t-test in the case of a normal distribution of values. For a distribution other than normal, the nonparametric Mann-Whitney test was used. The probability of an error-free forecast of qualitative signs was carried out using the Xi-square test (x2). When analyzing the data, the differences between the groups were considered significant at $p < 0.05$.

RESULTS

According to inclusion and exclusion criteria, 63 patients (52 men and 11 women) were included in the study, the average age of which was 57.3 (7.6) years. The average duration of COPD was 15 (3.6) years.

Depending on the smoking status, patients were divided into two groups: non-smoking patients — group I, smoking patients were included in group II (table I).

Group I included 26 patients (19 men and 7 women), whose average age was 55.8 (6.7) years. The severity of shortness of breath in accordance with the mMRS scale averaged 2 (1; 3) points, the frequency of exacerbations in the previous year was 1 (1; 2).

Group II consisted of 37 smoking patients (33 men and 4 women). The average age is 58.3 (8.1) years. The average severity score of dyspnea on the mMRS scale was 3 (2; 4) points, the frequency of exacerbations over the past year

Table III. Indicators of nutritional status in the examined patients with COPD.

Characteristics	Group I (non-smokers)	Group II (smokers)	p
Age, y M(SD)	55,8 (6,7)	58,3 (8,1)	0,1
Body mass , kg Me [25 %-75 %]	87,0 (82,0-88,0)	78,0(71,7-93,3)	0,7
BMI, Me [25 %-75 %]	26,3(25,0-30,0)	26,6(23,9-30,3)	0,9
Fat tissue, % Me [25 %-75 %]	25,05(24,6- 25,1)	35,1(31,1-37,5)	0,001
Muscle tissue , % Me [25 %-75 %]	39,9(34,5-44,9)	20,8 (16,8-29,7)	0,002
Visceral fat , % Me [25 %-75 %]	10,5 (8,0-12,0)	8,0 (5,5-11,0)	0,2
Waist circumference , sm M(SD)	95,5 (1,5)	91,5 (1,7)	0,3

Table IV. Laboratory characteristics of blood and urine of patients with COPD.

Characteristics	Group I (non-smokers)	Group II (smokers)	p
CRP , mg / l Me [25 %-75 %]	5,8 (2,6-11,6)	6,0 (2,4-10,8)	0,3
H, mmol / l Me [25 %-75 %]	4,6 (4,3-5,5)	4,6 (3,8-5,3)	0,4
LDL cholesterol, mmol / l Me [25 %-75 %]	2,9(2,3-3,6)	2,5(2,0-3,1)	0,045
VLDL , mmol / l Me [25 %-75 %]	0,5(0,4-0,7)	0,5(0,4-0,8)	0,6
HDL cholesterol, mmol / l Me [25 %-75 %]	1,1(1,0-1,3)	1,2(1,0-1,4)	0,3
TG, mmol / l Me [25 %-75 %]	1,1 (0,9-1,5)	1,2 (0,9-1,7)	0,9
AC Me [25 %-75 %]	3,0 (2,2-4,2)	2,8 (2,0-3,3)	0,1
Blood creatinine level , µmol / l Me [25 %-75 %]	92,0 (79,8-96,5)	84,8 (76,2-90,3)	0,6
GFR , ml / min Me [25 %-75 %]	76,0(72,5-82,1)	79,0 (74,2-83,0)	0,6
Urine creatinine level, µmol / l Me [25 %-75 %]	1078,4 (551,6)	1047, 6 (495,7)	0,02

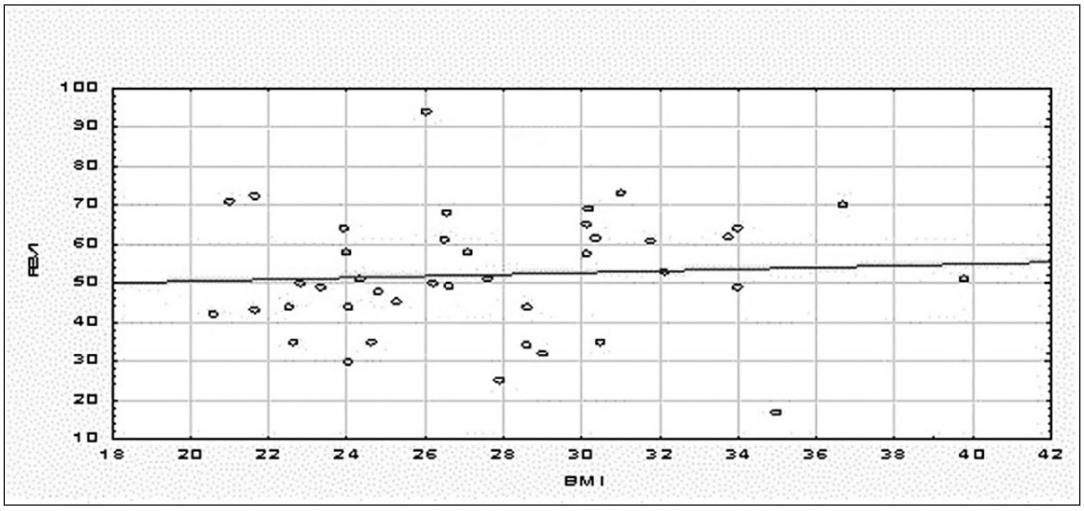

Fig. 1. The relationship between BMI and FEV1.

was 1 (1.3). The "pack / year" index was 29.8 (11.3). The degree of nicotine addiction according to the Fagerstrom test was 5.8 (1.8), which corresponded to the average level of dependence.

When comparing patients of both groups, there was no significant difference between the frequency of exacerbations by age, however, in smokers with COPD, the severity of shortness of breath was significantly higher (table I).

Significant differences confirming the high severity of bronchial obstruction in smokers with COPD were identified on the basis of spirometry data: for group I patients, the FEV1 index was 51.0 (44.0 -62.0)%, in group II - 45.0 (34.0 -59.0)% (p = 0.04) (table II).

Based on the conducted correlation analysis in patients of group II (smokers), an average negative correlation was found between the MOC 25 and the pack / year index (R = -0.5) (p <0.05).

Assessment of the nutritional status of patients was carried out on the basis of anthropometry and bioimpedancemetry data. BMI in patients of both groups did not

practically differ, despite a decrease in average body weight in patients of group II (smokers). Significant differences were found between the content of fat and muscle mass: in patients in the group of smokers, the percentage of total fat was significantly higher than in non-smokers. At the same time, the percentage of muscle tissue in smokers was significantly lower (table III).

Correlation analysis data indicate the presence of a positive correlation of moderate degree between the FEV1 and BMI (R = 0.4) in group II (smokers), between the indicator (FEV1%M) and the percentage of visceral fat (R = -0.7) (p <0.05) revealed a strong negative correlation.

A moderate correlation was found between the number of exacerbations and the content of muscle tissue in smokers with COPD (R = - 0.5) (p <0.05).

In the group of non-smokers, the level of LDL was 2.9 (2.3-3.6) mmol / L, in the group of smoking patients - 2.5 (2.0-3.1) mmol / L, (p = 0.045).

An average negative correlation between body weight and TG level (R = -0.68) (p <0.05) was also obtained.

Thus, in the examined patients, urine creatinine level in group I was 1078.4 (551.6) μmol / L, in group II - 1047.6 μmol / L (495.7) (p = 0.02).

Also, in all patients with COPD, a decrease in GFR below 90 ml / min was detected.

A strong negative correlation was found between urine creatinine level and the pack / year index (R = - 0.6) (p <0.05). A strong negative correlation between GFR and the degree of patients' compliance to smoking (R = - 0.7) was also determined.

DISCUSSION

In the course of the study, it was found that with an increase in the "pack / year" index in patients with COPD, the severity of bronchial obstruction increases, which confirms the literature [2]. Also, when studying the nutritional status, it was found that in patients with COPD who smoke, amid an increase in fat mass, a decrease in muscle mass was observed and sarcopenic obesity syndrome developed [15,16]. Our data confirm the literature data that in patients with smokers, against the background of a general decrease in body weight, primarily due to muscle mass, there is an increase in the content of visceral fat, which contributes to an increase in their degree of bronchial obstruction [16,17]. In smokers In patients, a decrease in muscle mass can contribute to an increase in the number of exacerbations of COPD, which is also reflected in the literature [6].

In patients with COPD there is a development of systemic effects and, above all, damage to the cardiovascular system, followed by the development of coronary artery disease, arterial hypertension, which, of course, is associated with changes in lipid metabolism, so the level of low density lipoproteins in patients of both groups exceeded the optimal level. It is of interest to identify a lower LDL content in smokers compared with non-smokers, which may be explained by the development in patients with COPD of systemic inflammation, more pronounced in smokers with COPD. Thus, an increase in CRP was detected in patients of both groups with a predominance of the indicator in the group of smoking patients. It is known that the development of systemic inflammation reduces the pool of muscle tissue. Probably, in this situation, muscle cells that need protection need cholesterol, while they synthesize LDL receptors, which, after synthesis, are transported to the cell membrane. In the cell membrane, LDL receptors are found in clathrin-containing caveoles. The LDL circulating in the blood binds to these transmembrane receptors and is endocytosed by the cell. After absorption, LDL is delivered to endosomes, and then to lysosomes, where cholesterol esters are hydrolyzed and cholesterol enters the cell [11, 16].

Visceral obesity is prognostically unfavorable in terms of the development of renal pathology [18]. There is scientific evidence supporting a link between abdominal obesity, microalbuminuria (MAU) and renal failure. The metabolic pathway for the development of glomerulosclerosis involves the deposition of low-density lipoproteins and apoliproteins B and E in the structures of the kidney. The accumulation of lipids in the renal tissue, as with atherosclerosis, induces sclerosis, which contributes to the development of renal pathology. Also, in all patients with COPD, a decrease in renal filtration function was detected. The effect of smoking on the development of metabolic disorders in the kidneys is evidenced by the obtained strong negative correlation between urine creatinine level and pack / year index: with an increase in smoking history, the percentage of muscle mass decreases, and, in turn, the blood and urine creatinine level, as creatinine is directly proportional to the amount of muscle tissue. A strong negative correlation between GFR and the degree of patients' compliance to smoking was also determined. Thus, in patients with COPD with nicotine dependence, there is a deterioration in renal function [12,19,20].

CONCLUSIONS

1. Patients suffering from COPD have a violation of nutritional status. Smoking patients develop sarcopenic obesity, which progresses with an increase in the degree of nicotine addiction, correlates with the "pack / year" index and is a predictor of increased mortality in this category of patients.
2. Increased bronchial obstruction in smokers with COPD is observed with an increase in smoking history, the number of cigarettes smoked and with a decrease in body weight.
3. Reducing the pool of muscle tissue can be considered as an early predictor of more frequent exacerbations in smoking patients with COPD.
4. The systemic effects of COPD include impaired renal function, more pronounced in smokers with COPD.

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ORIGINAL ARTICLE

MORPHOLOGICAL FEATURES OF CELLULAR INFILTRATION IN THE MUCOSA OF LARGE INTESTINE IN ULCERATIVE COLITIS AND IRRITABLE BOWEL SYNDROME

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ABSTRACT

The aim is to improve morphological diagnostics, including differential, of UC and IBS, identifying morphological features of cellular infiltration in the mucous membrane of the large intestine.

Material and methods: Autopsy and biopsy material – fragments of the mucous membrane of the large intestine was used in this study. All the material was divided into 5 groups. Group 1 included autopsy material from 6 cases, in which no general pathological processes in the gastrointestinal tract were detected during autopsy and microscopic examination. Group 2 included biopsy material from 34 patients with diagnosed UC of the 1st activity degree. Group 3 included the biopsy material of 27 patients with UC of the 2nd degree of activity. Group 4 included biopsy material from 19 patients, diagnosed with UC of the 3rd degree of activity. Group 5 included biopsy material from 82 patients with clinically diagnosed IBS. Histological, morphometrical, immunohistochemical and statistical methods of investigation were used.

Results: The mucous membrane of the large intestine in patients with ulcerative colitis of varying degrees of activity, compared with the physiological norm, has pronounced infiltration by plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes in the superficial parts of the epithelium, crypts, lamina propria. There is also an increase in the number and size of lymphoid follicles in the lamina propria. Predominant cellular elements in the infiltrate are plasma cells, T-lymphocytes, eosinophilic and neutrophilic leukocytes.

The growth of ulcerative colitis activity leads to an increase the inflammatory cell infiltration in the mucous membrane of the colon, as evidenced an increase the density of cellular infiltrate; the severity of inflammatory changes in crypts and an increase in the number of crypt abscesses; a decrease the number of cases with focal infiltration in the lamina propria and an increase the number of cases with diffuse infiltration; the spread of inflammatory cell infiltration from the superficial parts of the lamina propria to its deep parts with the subsequent involvement of its entire thickness; an increase the central trends of the indexes of the severity of all cellular infiltration, infiltration by plasma cells, T-lymphocytes, macrophages, neutrophilic leukocytes.

The mucous membrane of the large intestine in patients with irritable bowel syndrome has moderately pronounced cellular infiltration in the superficial epithelium and lamina propria, in comparison with the physiological norm. The number and size of lymphoid follicles increase. Inflammatory cell infiltration often spreads to the upper one third or two thirds of the thickness of the lamina propria, characterized by the presence of plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes. In this case, plasma cells, T-lymphocytes, mast cells and macrophages dominate. The indexes of the severity of all cellular infiltration, as well as infiltration by plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes, increases in the mucous membrane of the large intestine in irritable bowel syndrome in comparison with the norm.

In the mucous membrane of the large intestine in irritable bowel syndrome compared with ulcerative colitis of varying degrees of activity inflammatory cell infiltration is less pronounced. It often extends to one third or two thirds of the thickness of the lamina propria. There are fewer lymphoid follicles, cryptitis and crypt abscesses are not determined. The indexes of the severity of all cellular infiltration, as well as infiltration by plasma cells, T-lymphocytes, eosinophilic and neutrophilic leukocytes are lower.

Conclusions: The revealed features of cellular infiltration in the mucous membrane of the large intestine make it possible to improve morphological diagnostics, including differential, of ulcerative colitis of varying degrees of activity and irritable bowel syndrome.

KEY WORDS: ulcerative colitis, irritable bowel syndrome, large intestine mucosa, cellular infiltration, morphology

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INTRODUCTION

Ulcerative colitis (UC), being one of the most serious and unsolved problems of modern medicine, is characterized by inflammation of the colon mucosa, involving rectum, and possible retrograde spread of the inflammatory process to the proximal part of the ileum [1].

The UC prevalence in the world is 50-230 cases per 100 thousand population, the annual increase in patients is 5-20 cases per 100 thousand population. The highest incidence is in North America, Northern Europe and Australia, less often this pathology is recorded in Asia, South America and Japan. Among the white population, this pathology occurs 3-5 times more often than among African Americans, among Jews – 3.5 times more often than among non-Jewish people [2].

To date, etiology and pathogenesis of UC are not sufficiently studied. UC is characterized by a long course and

development of formidable complications. It leads to a high level of disability among young and mature people of working age, causing certain diagnostic difficulties for doctors, including during conducting differential diagnostics with IBS. The latter, according to the IV revision of the Rome criteria for functional disorders of the gastrointestinal tract, is determined as a functional disorder of the intestine. It is characterized by recurring abdominal pain at least once a week for the last 3 months, associated with the act of defecation and combined with changes in stool frequency and consistency of feces, with a total duration of complaints of at least 6 months [3].

Actualization of IBS diagnosing is determined by a decrease in the quality of patients' life, serious economic losses and epidemiological characteristics. IBS prevalence in the world is 11.2%. It is more common in women and, which is important, in young people [4].

Difficulties in the differential diagnosis of UC and IBS, as shown by numerous studies, are due to common clinical manifestations, genetic changes, disorders of the brain-intestinal axis, as well as disorders of the intestinal microbiota, etc. [5].

Thus, the question of finding the most significant criteria for differential diagnosis of UC and IBS remains relevant. An important role in the search for such diagnostic criteria belongs to colonoscopy with taking biopsy and its subsequent study, using various morphological research methods. Biopsy is considered a «gold» standard in the intravital diagnosis of intestinal pathology [6].

THE AIM

The aim is to improve morphological diagnostics, including differential, of UC and IBS, identifying morphological features of cellular infiltration in the mucous membrane of the large intestine.

MATERIAL AND METHODS

Autopsy and biopsy material – fragments of the mucous membrane of the large intestine was used in this study. All the material was divided into 5 groups. Group 1 included autopsy material from 6 cases, in which no general pathological processes in the gastrointestinal tract were detected during autopsy and microscopic examination. Group 2 included biopsy material from 34 patients with diagnosed UC of the 1st activity degree. Group 3 included the biopsy material of 27 patients with UC of the 2nd degree of activity. Group 4 included biopsy material from 19 patients, diagnosed with UC of the 3rd degree of activity. Group 5 included biopsy material from 82 patients with clinically diagnosed IBS. In patients in groups 2-4, UC was diagnosed based on clinical data, colonoscopy results and biopsy studies.

The resulting material was fixed in a 10% solution of neutral formalin (ph 7.4) for 24-48 hours, carried out according to the generally accepted technique and embedded in paraffin. From paraffin blocks on a rotational microtome HM 325 (Thermo Fisher Scientific, USA), serial sections with a thickness of 2 μm were made, stained with hematoxylin and eosin.

Immunohistochemical study was performed using rabbit monoclonal antibodies (MCA) to CD3 (clone SP7) (marker of T-lymphocytes), murine MCA to CD20 Ab-1 (clone L26) (marker of B-lymphocytes), murine MCA to CD138 Ab-2 (clone MI15) (marker of plasma cells), murine MCA to CD68 Ab-3 (clone KP1) (marker of macrophages), rabbit polyclonal antibodies (PCA) to CD117/c-Kit/SCF-Receptor (marker of mast cells). Ultra Vision Quanto HRP detection system was used in visualization. MCA, PCA, and imaging systems were manufactured by Thermo Fisher Scientific (USA).

Microscopic examination and photoarchiving were performed using light optical microscope Axio Imager. A2 (Carl Zeiss, Germany) with data processing system with magnified lenses ×5, ×10, ×20, ×40, binocular nozzle 1.5 and glasses 10 with ERc 5s camera, light optical microscope Primo Star (Carl Zeiss, Germany) with AxioCam 105 camera.

In each case, with a × 400 microscope magnification in 6 fields of view, the severity of the cellular infiltration (%) was assessed, using an adapted visual-analog scale [7].

The obtained digital data were processed, using the program Statistica 10.0. Central trends in the groups were compared on averages, using the nonparametric Mann-Whitney U test. Differences were considered significant at $p<0.05$, taking into account the Bonferroni correction [8].

RESULTS AND DISCUSSION

During the survey microscopy in group 1 there were a few, diffusely located cellular elements in the mucous membrane of the colon between the epithelial cells, in the lamina propria, mainly in its superficial parts. In some fields of view, cellular infiltration was not found, there were single lymphoid follicles. In this group the index of the severity of all cellular infiltration was (2.31±0.14)% (fig. 1).

During further analysis of hematoxylin and eosin stained microspecimens and the results of the immunohistochemical study it was noted that cellular infiltration in group 1 was represented by CD 3^{+}-cells, CD 138^{+}-cells, CD 20^{+}-cells, CD 68^{+}-cells, CD 117^{+}-cells, as well as eosinophilic and neutrophilic leukocytes. In this group, given the value of the index of the severity of cellular infiltration (table I), the 1st rank place was taken by T-lymphocytes, the 2nd place – by plasma cells, mast cells, macrophages, the 3rd place – by eosinophilic and neutrophilic leukocytes, B-lymphocytes. Thus, T-lymphocytes, plasma cells, mast cells and macrophages dominated in the cell infiltrate, identified in group 1.

Infiltration of immune cells, lymphoid follicles in the mucous membrane of the colon in group 1 are a variant of the norm, which agrees with the data of other scientists. It is known that the density and distribution of chronic inflammatory cells varies with anatomical site. Cellularity

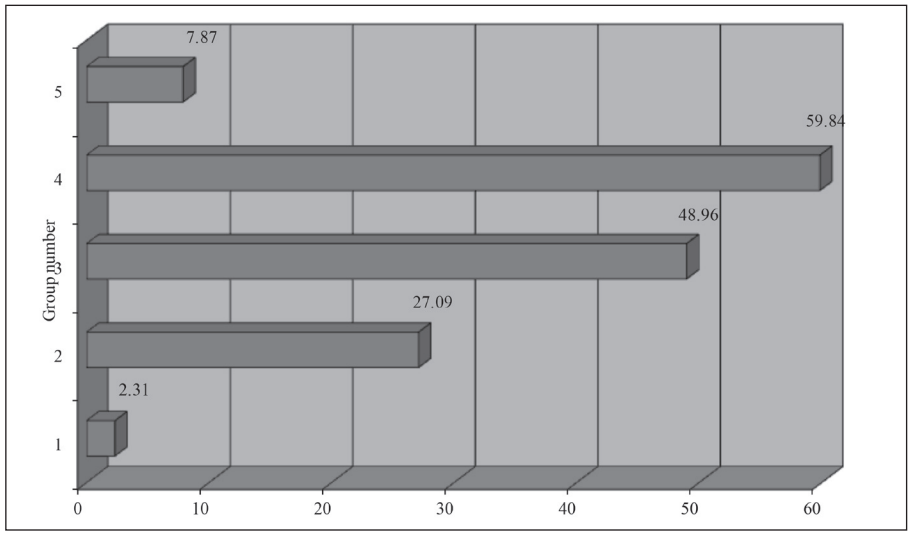

Fig. 1. Central trends of the indexes of the severity of all cellular infiltration (%) in groups 1-5.

Table I. Central trends of the indexes of the severity of infiltration by various cells (%) in groups 1-5.

Cells name	Group number				
	1	2	3	4	5
Plasma cells (CD 138)	3.69±0.12	30.03±0.96 [1, 3, 4, 5]	53.56±1.56 [1, 2, 4, 5]	63.63±1.92 [1, 2, 3, 5]	14.78±0.38 [1, 2, 3, 4]
T-lymphocytes (CD 3)	5.14±0.11	9.88±0.36 [1, 3, 4, 5]	16.26±0.63 [1, 2, 4, 5]	20.53±1.00 [1, 2, 3, 5]	7.78±0.29 [1, 2, 3, 4]
B-lymphocytes (CD 20)	0.47±0.07	1.91±0.17 [1, 4]	2.41±0.21 [1]	3.79±0.21 [1, 2, 5]	1.52±0.09 [1, 4]
Macrophages (CD 68)	1.48±0.17	3.97±0.14 [1, 3, 4]	5.93±0.34 [1, 2, 4]	8.89±0.33 [1, 2, 3, 5]	4.48±0.17 [1, 4]
Mast cells (CD 117)	2.74±0.15	5.21±0.19 [1, 4]	6.04±0.18 [1, 4]	8.11±0.21 [1, 2, 3, 5]	6.99±0.16 [1, 4]
Eosinophilic leukocytes	0.59±0.05	5.68±0.22 [1, 4, 5]	6.78±0.27 [1, 5]	7.32±0.31 [1, 2, 5]	3.70±0.10 [1, 2, 3, 4]
Neutrophilic leukocytes	0.24±0.04	5.65±0.21 [1, 3, 4, 5]	12.30±0.69 [1, 2, 4, 5]	24.95±1.15 [1, 2, 3, 5]	1.09±0.08 [1, 2, 3, 4]

[1] – differences are significant compared to the index of group 1,
[2] – differences are significant compared to the index of group 2,
[3] – differences are significant compared to the index of group 3,
[4] – differences are significant compared to the index of group 4,
[5] – differences are significant compared to the index of group 5.

is highest in the caecum/ascending colon and may be low in the rectum [9, 10].

The immune system of the gastrointestinal tract, as is known, is located in the mucous membrane and is in close contact with a huge flow of microbial and allergenic material, coming from the intestinal lumen. It serves as the first barrier on its way [11]. A similar infiltration by immune cells is also found in the mucous membrane of the respiratory system, urinary system, etc. [12].

In patients with UC of various degrees of activity (groups 2-4), compared with group 1, in the mucous membrane of the colon the number and size of lymphoid follicles increased, cell infiltration was more pronounced and increased from group 2 to group 4, as evidenced by survey microscopy (fig. 2, 3), analysis of the indexes of the severity of cell infiltration, an immunohistochemical study.

Observational microscopy in groups 2-4 revealed inflammatory cell infiltration in the superficial epithelium, crypt lumen, which indicated cryptitis development and crypt abscesses formation. Presence of cryptitis and crypt abscesses is a characteristic microscopic sign of active UC, which we noted in our previous studies and the works of other scientists [9, 13]. It was determined that with an increase in the degree of UC activity, inflammatory changes in crypts increased and the number of crypt abscesses increased.

In groups 2-4, inflammatory cell infiltration was also found in the lamina propria of the colon mucosa, where it was irregular, focal, or diffuse, when cellular infiltrates formed continuous fields. In group 2, focal and diffuse

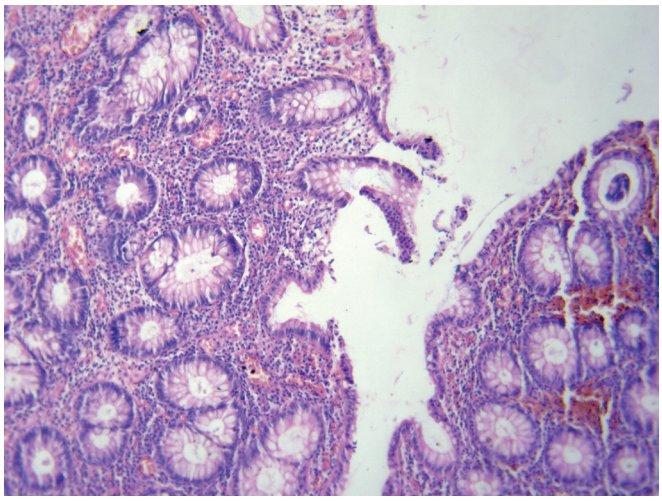

Fig. 2. The mucous membrane of the large intestine of a patient with UC of group 2. Stained with hematoxylin and eosin, × 100.

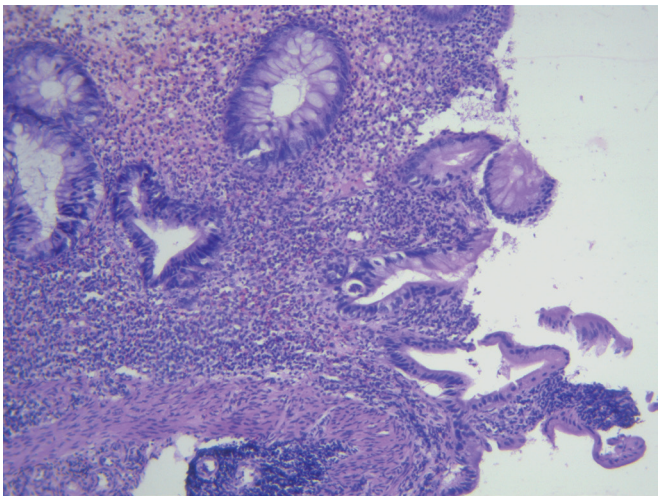

Fig. 3. The mucous membrane of the large intestine of a patient with UC of group 4. Stained with hematoxylin and eosin, × 100.

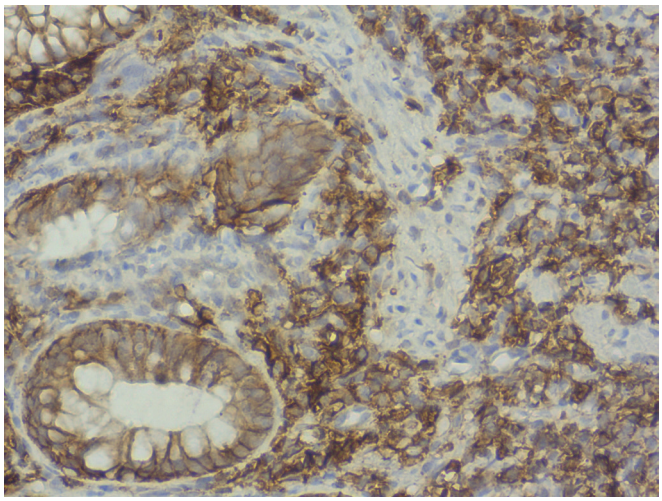

Fig. 4. CD 138^{+}-cells in the mucous membrane of the colon of a patient with UC of group 4. Immunohistochemical reaction with MCA to CD 138 Ab-2, × 400.

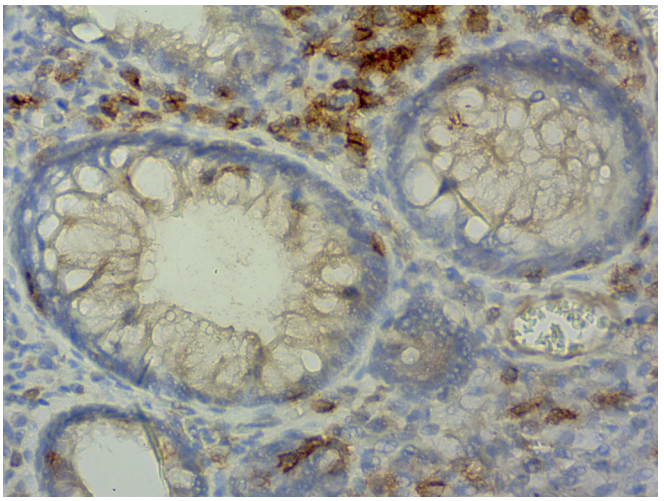

Fig. 5. CD 3^{+}-cells in the mucous membrane of the colon of a patient with UC of group 2. Immunohistochemical reaction with MCA to CD 3, × 100.

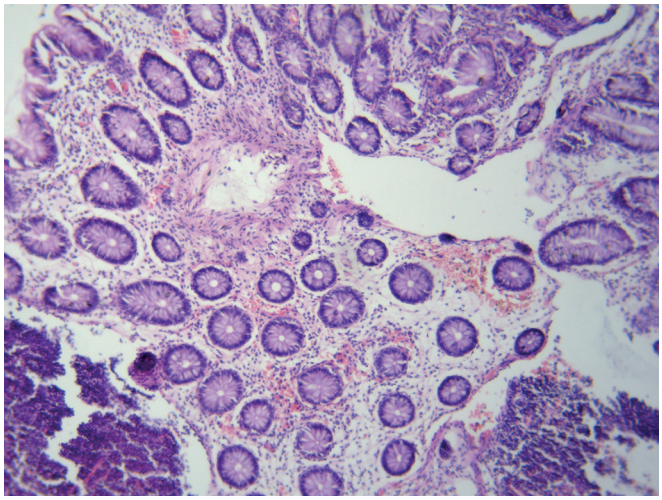

Fig. 6. The mucous membrane of the large intestine of a patient with IBS of group 5. Stained with hematoxylin and eosin, × 100.

cellular infiltration was detected in 25 (73.5%) and 9 (26.5%) cases, respectively. In group 3 in 16 (59.3%) cases focal infiltration was noted, and in 11 (40.7%) – diffuse. In group 4, inflammatory cell infiltration was focal in 5 cases (26.3%) and diffuse in 14 (73.7%). Thus, with an increase in UC activity, cell infiltrate density increased, the number of cases with focal infiltration in the lamina propria of the colon mucosa decreased and the number of cases with diffuse infiltration increased.

Under survey microscopy in groups 2-4 in the lamina propria of the colon mucosa, inflammatory cell infiltration was determined in its upper third, occupied two thirds, or spread to its entire thickness. In 2 group, inflammatory cell infiltration in the lamina propria of the mucous membrane in 2 cases (5.9%) was located in its upper third, and in 23 (67.6%) and 9 (26.5%) cases it spread to its two-thirds of the thickness and all its thickness. In group 3, inflammatory infiltration in the lamina propria of the mucous membrane

was located in 12 cases (44.4%) in its two-thirds of the thickness, and in 15 cases (55.6%) in its entire thickness. In group 4, cellular infiltration in the lamina propria was located in its two-thirds and its entire thickness, respectively, in 6 (31.6%) and 13 (68.4%) cases. Thus, with an increase in UC activity, inflammatory cell infiltration spread from the superficial parts of the lamina propria to its deep parts, with the subsequent involvement of its entire thickness.

The index of the severity of all cellular infiltration in the lamina propria of the colon mucosa in groups 2-4 acquired a significantly ($p<0.05$) higher value than that of group 1 and increased ($p<0.05$) from group 2 to group 4 (fig. 1).

In patients of groups 2-4, inflammatory cell infiltration in the mucous membrane of the colon was represented, as in group 1, by plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes.

Plasma cells were found in the cellular infiltrate throughout the entire thickness of the mucous membrane of the large intestine. They were located focal or diffusely (fig. 4). These cells were often found in small numbers in lymphoid follicles, near crypts or under crypts. It is known that basal plasmacytosis, especially when combined with crypt deformation, is a pathognomonic microscopic sign of UC [14].

T-lymphocytes were localized more often diffusely in the entire thickness of the lamina propria of the colon mucosa. Also, these cells were found between some epithelial cells and in lymphoid follicles (fig. 5).

B-lymphocytes localized focally, mainly in the entire thickness of the lamina propria of the colon mucosa around or directly in the lymphoid follicles.

Macrophages were characterized by focal or diffuse location, both in the superficial and in the deep parts of the lamina propria of the mucous membrane. Cells of the macrophage series were often located pericriptally or in places of erosive and ulcerative changes.

In the cellular infiltrate, mast cells were more often characterized by a diffuse arrangement and eosinophilic leukocytes by a focal arrangement. Infiltration by mast cells and eosinophilic leukocytes spread to deep sections or the entire thickness of the lamina propria of the mucous membrane.

Neutrophilic leukocytes in the cell infiltrate in patients of group 2 more often localized diffusely in the superficial parts of the lamina propria of the mucous membrane. With an increase in the degree of UC activity, these cells spread to the deep parts of the lamina propria, and in patients of group 4 covered its entire thickness.

Analyzing the values of central trends of the indexes of the severity of infiltration by various cells (table I) in group 2 have shown the 1st rank place for plasma cells, the 2nd – T-lymphocytes, the 3rd – eosinophilic and neutrophilic leukocytes, mast cells, the 4th – macrophages and B-lymphocytes. In group 3, the 1st rank place was allocated for plasma cells, the 2nd place – T-lymphocytes, the 3rd place – neutrophilic leukocytes, the 4th place – eosinophilic leukocytes, mast cells, macrophages, the 5th place – B-lymphocytes. In group 4, the 1st rank place was for plasma cells, the 2nd place – neutrophilic leukocytes, the 3rd place – T-lymphocytes, the 4th place – macrophages, mast cells and eosinophilic leukocytes, the 5th place – B-lymphocytes. Thus, in groups 2-4, plasma cells, T-lymphocytes, eosinophilic and neutrophilic leukocytes prevailed in the cellular infiltrate.

In groups 2-4, in comparison with group 1, the indexes of the severity of infiltration by various cells had significantly ($p<0.05$) larger values (table I). With an increase in UC activity, the index of the severity of infiltration by plasma cells, T-lymphocytes, macrophages and neutrophilic leukocytes increased ($p<0.05$). The index of the severity of infiltration by B-lymphocytes and eosinophilic leukocytes tended to increase ($p>0.05$) in group 3 as compared with group 2, in group 4 compared with group 3, and in group 4 compared with group 2 it increased ($p<0.05$). The index of the severity of infiltration by mast cell tended to increase ($p>0.05$) in group 3 compared to group 2, increased ($p<0.05$) in group 4 compared to group 3.

Under survey microscopy of the colon mucosa in patients with IBS of group 5, compared with group 1, revealed more pronounced inflammatory cell infiltration in the superficial epithelium and lamina propria, as well as lymphoid follicles in the lamina propria (fig. 6).

In group 5, in the lamina propria of the colon mucosa in 60 cases (73.2%) inflammatory cell infiltration was of a focal character, and in 22 cases (26.8%) it was diffuse. Inflammatory infiltration was more often located in one third or two third thickness layers of the lamina propria of the mucous membrane (in 22 cases (26.8%) – in its one third of the thickness, in 59 cases (72.0%) – in its two third, in 1 case (1.2%) – in its entire thickness). The index of the severity of all cellular infiltration in this group had a significantly ($p<0.05$) greater value than that of group 1 (fig. 1).

Cellular infiltration, revealed by observation microscopy in group 5, was characterized by a composition similar to group 1 and was represented by plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes.

In the lamina propria of the colon mucosa, plasma cells were often diffusely localized and occupied its upper two third of the thickness. Predominantly diffuse infiltration by T-lymphocytes, spreading to the upper two thirds of the lamina propria thickness of the mucous membrane, was determined. Focal infiltration with B-lymphocytes was found mainly in the entire thickness of the lamina propria of the colon mucosa around or directly in the lymphoid follicles. Macrophage infiltration and infiltration with eosinophilic leukocytes often had a focal character, and spread to the entire thickness of the lamina propria of the mucous membrane. Mast cells were often characterized by a diffuse arrangement in the cellular infiltrate, spreading to two thirds or the entire thickness of the lamina propria of the mucous membrane. Neutrophilic leukocytes in the cell infiltrate were found in 66 cases (80.5%) only in the upper third of the lamina propria of the mucous membrane.

In group 5, the indexes of the severity of infiltration by plasma cells, T-lymphocytes, B-lymphocytes, macro-

phages, mast cells, eosinophilic and neutrophilic leukocytes were significantly ($p<0.05$) higher, compared with similar indexes in group 1 (table I).

Analyzing central trends of the indexes of the severity of infiltration by various cells (table I) in group 5, plasma cells ranked the 1st place, T-lymphocytes and mast cells – the 2nd, macrophages, eosinophilic leukocytes – the 3rd, B-lymphocytes, neutrophilic leukocytes – the 4th. Thus, in this group the predominant cells in the infiltrate were plasma cells, T-lymphocytes, mast cells, macrophages.

Most scientists also noticed inflammation signs in the lamina propria of the colon mucosa in patients with IBS [15]. The inflammatory cellular infiltration in patients with IBS, according to the literature, is characterized by a predominantly small number of macrophages, lymphocytes, monocytes, plasma and mast cells, sometimes with an admixture of eosinophilic leukocytes [16]. These changes in the cellular composition of the colon mucosa in patients with IBS lead to the increased production of proinflammatory cytokines (TNFα, IL-8, IL-6, IL-1β, etc.). There were also significant changes in the composition of cells not only at the local (in the intestine), but also at the systemic level [17].

Comparative analysis of the data, obtained in groups 2-5, made it possible to reveal the distinctive signs of cellular infiltration in the mucous membrane of the large intestine in patients with UC of varying degrees of activity and IBS.

Firstly, in IBS compared with UC of varying degrees of activity in the mucous membrane of the colon inflammatory cell infiltration was less pronounced, more often it was localized in one third or two thirds of the lamina propria. There were fewer lymphoid follicles, cryptitis and crypt abscesses were not detected, the index of the severity of all cellular infiltration had a significantly ($p<0.05$) lower value.

Secondly, inflammatory cell infiltration in UC of varying activity degrees and IBS were characterized by the presence of plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes. However, plasma cells, T-lymphocytes, eosinophilic and neutrophilic leukocytes predominated in the infiltrate in UC, while in IBS plasma cells, T-lymphocytes, mast cells and macrophages were predominant.

Thirdly, in IBS compared with UC of varying activity degrees the indexes of the severity of infiltration by plasma cells, T-lymphocytes, eosinophilic and neutrophilic leukocytes were significantly ($p<0.05$) lower (table I). The indexes of the severity of infiltration by B-lymphocytes, macrophages, mast cells in IBS were not significantly ($p>0.05$) different compared with the corresponding indicators in the 1st and 2nd degrees of activity UC, but they had significantly ($p<0.05$) lower values compared to indicators for UC of the 3rd degree of activity.

CONCLUSIONS

1. The mucous membrane of the large intestine in patients with ulcerative colitis of varying degrees of activity, compared with the physiological norm, has pronounced infiltration by plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes in the superficial parts of the epithelium, crypts, lamina propria. There is also an increase in the number and size of lymphoid follicles in the lamina propria. Predominant cellular elements in the infiltrate are plasma cells, T-lymphocytes, eosinophilic and neutrophilic leukocytes.
2. The growth of ulcerative colitis activity leads to an increase the inflammatory cell infiltration in the mucous membrane of the colon, as evidenced an increase the density of cellular infiltrate; the severity of inflammatory changes in crypts and an increase in the number of crypt abscesses; a decrease the number of cases with focal infiltration in the lamina propria and an increase the number of cases with diffuse infiltration; the spread of inflammatory cell infiltration from the superficial parts of the lamina propria to its deep parts with the subsequent involvement of its entire thickness; an increase the central trends of the indexes of the severity of all cellular infiltration, infiltration by plasma cells, T-lymphocytes, macrophages, neutrophilic leukocytes.
3. The mucous membrane of the large intestine in patients with irritable bowel syndrome has moderately pronounced cellular infiltration in the superficial epithelium and lamina propria, in comparison with the physiological norm. The number and size of lymphoid follicles increase. Inflammatory cell infiltration often spreads to the upper one third or two thirds of the thickness of the lamina propria, characterized by the presence of plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes. In this case, plasma cells, T-lymphocytes, mast cells and macrophages dominate. The indexes of the severity of all cellular infiltration, as well as infiltration by plasma cells, T-lymphocytes, B-lymphocytes, macrophages, mast cells, eosinophilic and neutrophilic leukocytes, increases in the mucous membrane of the large intestine in irritable bowel syndrome in comparison with the norm.
4. In the mucous membrane of the large intestine in irritable bowel syndrome compared with ulcerative colitis of varying degrees of activity inflammatory cell infiltration is less pronounced. It often extends to one third or two thirds of the thickness of the lamina propria. There are fewer lymphoid follicles, cryptitis and crypt abscesses are not determined. The indexes of the severity of all cellular infiltration, as well as infiltration by plasma cells, T-lymphocytes, eosinophilic and neutrophilic leukocytes are lower.
5. The revealed features of cellular infiltration in the mucous membrane of the large intestine make it possible to improve morphological diagnostics, including differential, of ulcerative colitis of varying degrees of activity and irritable bowel syndrome.

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ORIGINAL ARTICLE

RELATIONSHIP BETWEEN PATHOLOGICAL MENSTRUAL SYMPTOMS AND THE DEVELOPMENT OF EXTRAGENITAL FORMS OF LOCAL INFLAMMATION

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ABSTRACT

The aim: To evaluate the parameters of menstrual function in 1015 women of reproductive age and to establish the relationship between the detected menstrual disorders and the development of migraine and/or irritable bowel syndrome (IBS).

Materials and methods: During 2018–2020, a survey of women of reproductive age in the Ternopil region (Ukraine) was conducted. To interview the study respondents, we developed a questionnaire that assessed the parameters of menstrual function (age of menarche; regularity and cyclicity of menstrual changes; duration of the menstrual cycle and menstruation itself; the amount of blood loss with the pictogram, the presence of clots and their size) identify characteristic changes in health, symptoms of irritable bowel syndrome and migraine associated with the menstrual cycle. Data analysis was performed by statistical and mathematical method.

Results and conclusions: In 72.2 % of respondents, the study revealed deviations from the normal course of the menstrual cycle. In particular, an increase in blood loss during menstruation and the appearance of clots larger than 1 cm were observed in 40.8 % of women. Among patients with menstrual dysfunction, 51.8 % of patients had symptoms of irritable bowel syndrome, and 44.1 % had signs of migraine.

Thus, the relationship between cyclic menstrual disorders and the formation of IBS and migraine, which significantly affects the deterioration of health, performance of women of childbearing age.

KEY WORDS: menstrual cycle and its features, menstrual inflammation, irritable bowel syndrome (IBS), migraine disorders, women of reproductive age

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INTRODUCTION

In recent years, in the modern medical literature there are frequent data on the relationship between disorders of endometrial regeneration in menstrual cycles and the emergence of inflammatory symptoms in organs with genetically determined or acquired sensitivity to monthly hormonal and immunological changes in the reproductive organs [1, 2].

In particular, the study by Bertone-Johnson E.R., Ronnenberg A.G., Houghton S.C., et al. [3], in patients of reproductive age with manifestations of premenstrual syndrome (PMS) there were pronounced fluctuations in the concentrations of key inflammatory mediators (cytokines – interleukins of the types 8, 6 and 4; matrix metalloproteinase-1; cyclooxygenase-2, prostaglandins PGE2 and PGF2α, histamine, serotonin, bradykinin, tumor necrosis factor-α) during the cycle compared with women without PMS [4, 5].

According to the works of Heitkemper M.M. et al. and Berbic M., Ng C.H.M., Fraser I.S., who studied immunological changes in patients with normal menstrual function, menstrual inflammation should be considered as a physiological process that occurs in response to interactions between the endocrine and immune systems [6–8]. However, the appearance of symptoms of local and systemic inflammation in the non-reproductive organs and systems associated with menstruation is the result of a violation of the latter [9, 10, 11].

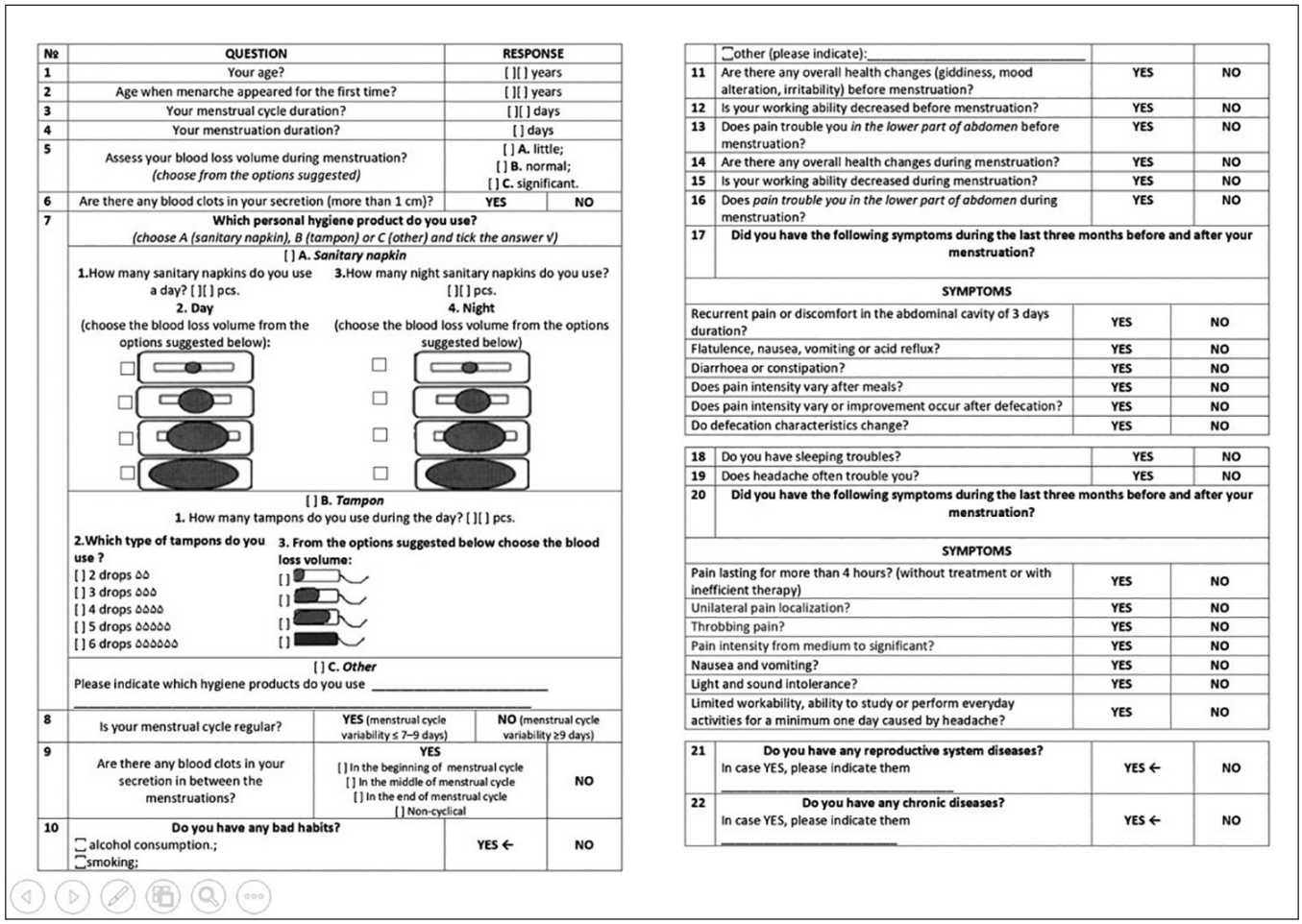
Thus, further study of the features of the menstrual cycle as a predictor of the development of pathological disorders in organs and systems genetically sensitive to cyclical changes in the body of a woman is relevant.

THE AIM

The aim of the study was to evaluate the parameters of menstrual function in 1015 women of reproductive age and to establish the relationship between the detected menstrual disorders and the development of migraine and/or irritable bowel syndrome.

MATERIALS AND METHODS

The study was conducted on the basis of the Ternopil Regional Clinical Perinatal Center "Mother and Child", which is the clinical base of the Department of Obstetrics and Gynecology No. 1, Medical Faculty of Ternopil National Medical University.



№	QUESTION	RESPONSE	
1	Your age?	[ ][ ] years	
2	Age when menarche appeared for the first time?	[ ][ ] years	
3	Your menstrual cycle duration?	[ ][ ] days	
4	Your menstruation duration?	[ ] days	
5	Assess your blood loss volume during menstruation? *(choose from the options suggested)*	[ ] **A.** little; [ ] **B.** normal; [ ] **C.** significant.	
6	Are there any blood clots in your secretion (more than 1 cm)?	YES	NO
7	**Which personal hygiene product do you use?** *(choose A (sanitary napkin), B (tampon) or C (other) and tick the answer √)*		

[ ] **A.** ***Sanitary napkin***

1.How many sanitary napkins do you use a day? [ ][ ] pcs.

2. Day

(choose the blood loss volume from the options suggested below):

3.How many night sanitary napkins do you use? [ ][ ] pcs.

4. Night

(choose the blood loss volume from the options suggested below)

[ ] **B.** ***Tampon***

1. How many tampons do you use during the day? [ ][ ] pcs.

2.Which type of tampons do you use ?

[ ] 2 drops ∆∆
[ ] 3 drops ∆∆∆
[ ] 4 drops ∆∆∆∆
[ ] 5 drops ∆∆∆∆∆
[ ] 6 drops ∆∆∆∆∆∆

3. From the options suggested below choose the blood loss volume:

[ ] [ ] [ ] [ ]

[ ] **C.** ***Other***

Please indicate which hygiene products do you use _____

8	Is your menstrual cycle regular?	**YES** (menstrual cycle variability ≤ 7–9 days)	**NO** (menstrual cycle variability ≥9 days)
9	Are there any blood clots in your secretion in between the menstruations?	**YES** [ ] In the beginning of menstrual cycle [ ] In the middle of menstrual cycle [ ] In the end of menstrual cycle [ ] Non-cyclical	**NO**
10	**Do you have any bad habits?** [ ] alcohol consumption.; [ ] smoking;	**YES ←**	**NO**

	[ ] other (please indicate):_____		
11	Are there any overall health changes (giddiness, mood alteration, irritability) before menstruation?	YES	NO
12	Is your working ability decreased before menstruation?	YES	NO
13	Does pain trouble you *in the lower part of abdomen* before menstruation?	YES	NO
14	Are there any overall health changes during menstruation?	YES	NO
15	Is your working ability decreased during menstruation?	YES	NO
16	Does *pain trouble you in the lower part of abdomen* during menstruation?	YES	NO
17	**Did you have the following symptoms during the last three months before and after your menstruation?**		

SYMPTOMS		
Recurrent pain or discomfort in the abdominal cavity of 3 days duration?	YES	NO
Flatulence, nausea, vomiting or acid reflux?	YES	NO
Diarrhoea or constipation?	YES	NO
Does pain intensity vary after meals?	YES	NO
Does pain intensity vary or improvement occur after defecation?	YES	NO
Do defecation characteristics change?	YES	NO

18	Do you have sleeping troubles?	YES	NO
19	Does headache often trouble you?	YES	NO
20	**Did you have the following symptoms during the last three months before and after your menstruation?**		

SYMPTOMS		
Pain lasting for more than 4 hours? (without treatment or with inefficient therapy)	YES	NO
Unilateral pain localization?	YES	NO
Throbbing pain?	YES	NO
Pain intensity from medium to significant?	YES	NO
Nausea and vomiting?	YES	NO
Light and sound intolerance?	YES	NO
Limited workability, ability to study or perform everyday activities for a minimum one day caused by headache?	YES	NO

21	**Do you have any reproductive system diseases?** In case YES, please indicate them _____	YES ←	NO
22	**Do you have any chronic diseases?** In case YES, please indicate them _____	YES ←	NO

Fig. 1. Sample questionnaire.

Parameter	Normal	Abnormal	☑
Frequency	Absent (no bleeding) = amenorrhea		☐
	Infrequent (>38 days)		☐
	Normal (≥24 to ≤38 days)		☐
	Frequent (<24 days)		☐
Duration	Normal (≤8 days)		☐
	Prolonged (>8 days)		☐
Regularity	Normal or "Regular" (shortest to longest cycle variation: ≤7-9 days)*		☐
	Irregular (shortest to longest cycle variation: ≥8-10 days)*		☐
Flow Volume (patient determined)	Light		☐
	Normal		☐
	Heavy		☐
Intermenstrual Bleeding (IMB) Bleeding between cyclically regular onset of menses	None		☐
	Random		☐
	Cyclic (Predictable)	Early Cycle	☐
		Mid Cycle	☐
		Late Cycle	☐
Unscheduled Bleeding on Progestin ± Estrogen Gonadal Steroids (birth control pills, rings, patches or injections)	Not Applicable (not on gonadal steroid medication)		☐
	None (on gonadal steroid medication)		☐
	Present		☐

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Fig. 2. System for determining the parameters of normal menstrual blood loss (FIGO, 2018) [10].

In the period from 2018 to 2020, 1015 women were interviewed. The mean age of patients was (25.2±7.3) years.

To survey the respondents, we developed a questionnaire, which consisted of four blocks of questions. The first block was devoted to assessing the parameters of the menstrual cycle: the time of menarche, regularity and cyclicity, the duration of the cycle and menstruation itself, determining the amount of blood loss, taking into account the amount of hygiene products used day and night and the presence of clots. The presence of bad habits was assessed by question No. 10; changes in health and performance before and during menstruation – questions No. 11–16. The answers of the third and fourth blocks revealed symptoms of irritable bowel syndrome and migraine according to the recommendations of Roman criteria IV and the International Headache Society (Fig. 1) [12, 13, 14].

The assessment of menstrual cycle parameters was performed according to the updated in 2018 international guidelines (clinical guidelines of the National Institute of Health and Quality of Health Care of the United Kingdom (NICE) and the International Federation of Obstetricians and Gynecologists (FIGO)) (Fig. 2) [15]. Severe menstrual bleeding is considered an indicator of menstrual blood loss 80 ml (Clinical protocol for the management of patients with abnormal uterine bleeding, implemented in accordance with the order of the Ministry of Health of Ukraine of April 13, 2016 No. 353) [16].

For the reliability of the evaluation of the obtained data, the study group did not include patients who regularly (for more than 1 month) taking hormonal contraception and women with tumors and inflammatory diseases of the pelvic organs.

Statistical data processing was performed using Microsoft Excel and STATISTICA 12 software.

RESULTS

According to the questionnaire data, in 91.7 % of cases (931 patients) there was a normal duration of the menstrual cycle. A decrease of <24 days was found in 63 women (6.2 %) (average cycle duration (20.2±0.3) days), and an increase over 38 days – in 21 (2.1 %) (with an average cycle duration of (47.8±1.98) days).

Physiological duration of menstruation was diagnosed in 953 study participants, however, 58 women (5.7 %) indicated a duration of less than 4 days, and 4 (0.4 %) – more than 8 days.

Analysis of the menstrual blood self-monitoring pictogram showed an increase in blood loss in 40.1% of patients (407) and a mean volume was (156.9±5.33) ml.

It was noteworthy that among the cases of increased blood loss in 97.8 % of women there was a normal duration of menstruation, and in 90.9 % – the physiological duration of the menstrual cycle.

Underestimation of blood loss during menstruation in the form of blood clots larger than 1 cm was found in 188 patients with normal pictogram data, indicating chronic monthly blood loss outside of physiological.

In 75.6 % of respondents the menstrual cycle was regular, 24.4% – irregular. Intermenstrual bleeding bothered 8.6 % of women.

77 patients reported smoking, 61 – alcohol use, 69 – had both bad habits.

The answers of the participants showed that in most (977 women – 96.3 %) cyclic changes in the body were accompanied by malaise. Most often, patients complained of lower abdominal pain during menstruation, dizziness, mood lability, irritability. These complaints were prevalent in 97.3 % of women with increased blood loss during menstruation according to self-assessment and in 93.1 % of respondents who indicated the presence of clots of 1 cm or more during menstruation.

In the analysis process of the survey results, 47.9 % (486 women) showed manifestations of irritable bowel syndrome (IBS). Among 6 questions of the questionnaire regarding the diagnostic criteria of IBS (according to Roman Criteria IV), the affirmative answer in most cases concerned complaints about changes in the nature of bowel movements (63 %) and changes in pain intensity after defecation (63.6 %). 212 patients (43.6 %) with manifestations of IBS were characterized by complaints of increased menstrual bleeding over 80 ml, and 224 (46.1 %) – the presence of clots on critical days. The combination of symptoms of IBS with dysmenorrhea was observed in 84.2 % of women, with premenstrual syndrome – in 75.5 %.

According to the questionnaire, in 40.3 % of cases (409 women) there were signs of migraine, which were detected in the presence of three positive responses to the questionnaire (recommendations of the International Headache Association). Manifestation of headache symptoms was accompanied by menstrual irregularities: 188 (46 %) women had a blood loss of more than 80 ml; 180 respondents (44 %) of the study indicated the presence of clots larger than 1 cm; in 14 (3.4 %) women – the duration of the cycle ranged from more than 38 days, in 27 (6.6 %) – less than 24 days; 22 patients (5.4 %) indicated a duration of menstruation of less than 4 days, and 9 (2.2 %) – more than 8 days; irregular cycle was noted by 292 women (71.4 %).

In 115 patients with increased blood loss and in 114 cases of blood clots larger than 1 cm during menstruation, manifestations of IBS and/or migraine were detected.

DISCUSSION

According to the results of the study, 65.7 % of women of reproductive age had symptoms of IBS and/or migraine: IBS – in 486 women, migraine – in 409, combined pathology – in 228. Against the background of these symptoms, according to personal data, respondents had certain disorders menstrual cycle: in 78.2 % (370 women) with manifestations of IBS and in 79 % (323 patients) – migraines. The most common menstrual cycle disorders in patients with IBS and migraine were associated with changes in menstrual blood volume parameters (> 80 ml) and/or the appearance of clots larger than 1 cm on menstrual days: in IBS – 319 cases (65.6 %), with migraine – 264 (64.5 %), respectively.

Despite the fact that menstruation is a monthly physiological process of endometrial recovery in non-pregnant women of reproductive age, increasing the duration and intensity of bleeding over time leads to prolongation of local and systemic inflammatory reactions at different levels, which eventually are present in premenstrual syndrome (PMS) to premenstrual magnification.

Given the average age of menarche and the duration of the reproductive period in study respondents and cyclical abnormalities of the menstrual cycle, including increased menstrual blood loss, symptoms of IBS and migraine as manifestations of extragenital local inflammation, may be the result of inadequate restoration of normal cytoarchitecture of endometrial tissue on the background of immunological and hormonal changes.

CONCLUSIONS

1. The frequency of menstrual disorders among women in the study group was observed in 72.2 %. Among the latter, the symptoms of IBS were found in 51.8 %, migraines – in 44.1 %.
2. Among the menstrual cycle disorders in women with IBS most often there was a change in the parameters of menstrual blood volume> 80 ml (43.6 %) and the appearance of clots larger than 1 cm on critical days (46.1 %) and in patients with migraine – 46 % and 44 % respectively.
3. According to the results of the study, cyclic menstrual disorders with a pronounced increase in blood loss during menstruation should be considered as a trigger for the development of IBS and migraine.
4. Given this hypothesis, the management of the diagnosis of IBS and migraine in women of reproductive age should be supplemented by a detailed analysis of menstrual function, and treatment – to include appropriate correction of menstrual cycle disorders.

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ORIGINAL ARTICLE

LEVELS OF PROINFLAMMATORY CYTOKINES IL-17 AND IL-23 IN PATIENTS WITH ALZHEIMER'S DISEASE, MILD COGNITIVE IMPAIRMENT AND VASCULAR DEMENTIA

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ABSTRACT

The aim: To research differences of interleukin (IL)-17 and IL-23 serum levels in patients with Alzheimer's disease, vascular dementia and mild cognitive impairment.

Material and methods: Serum levels of IL-17 and IL-23 were measure by ELISA for 15 patients with Alzheimer's disease, 14 with vascular dementia, 30 with mild cognitive impairment and 30 control individuals without cognitive impairment.

Results: Serum concentrations of IL-17 were significantly higher in Alzheimer's disease patients (P=0.0023) than control, in vascular dementia no significant differences (P=0.4154). Level of IL-23 was significantly higher than control in Alzheimer's disease patients (P=0.0170) and vascular dementia (P=0.0002), but in Alzheimer's disease it was in 12.5 time higher. In total mild cognitive impairment patients no significant differences in interleukin concentration with control, but significant differences observed for amnestic form in IL-17 (P=0.0436) and IL-23 (P=0.0019).

Conclusions: IL-17 and IL-23 level significant higher in Alzheimer's disease patients compared with control and vascular dementia. From mild cognitive impairment levels of detectable interleukins was higher in amnestic form that may be early marker of progression in Alzheimer's disease.

KEY WORDS: Alzheimer's disease, vascular dementia, interleukins

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INTRODUCTION

Among old-related dementia, Alzheimer's disease (AD) is the most common and characterized by a progressive and irreversible deterioration of cognitive and function abilities [1]. Dementia was name major neurocognitive disorder (NCD) in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [2]. Mild NCD is a diagnostic category in DSM-5 added to recognize the substantial clinical need of individuals living with this disorder, which might also be termed mild cognitive impairment (MCI). Mild NCD possible is preddementia stage in AD but not always a precursor of major NCD. AD is a multifactorial etiopathogenesis disorder and neuroinflammatory processes are a central feature in which microglia are over-activated, resulting in increased production of pro-inflammatory cytokines. Evidence suggests that different cytokines, including interleukins (IL) IL-6, IL-10, IL-12, TNF-α and TGF-β are actively participated in AD pathogenesis [3]. IL-17 and IL-23 augmented in AD patients upon stimulating of cell with Aβ in vitro and play role in AD-associated neuroinflammation [4].

THE AIM

The aim of this study to research differences of interleukin (IL)-17 and IL-23 serum levels in patients with Alzheimer's disease, vascular dementia and mild cognitive impairment.

MATERIALS AND METHODS

The study involved 59 patients with cognitive impairment (43 men and 46 women, average age – 66.8 ± 8.4 years), of which 29 has major NCD and 30 mild NCD. 15 (25.4%) patients with major NCD meet to updated criteria for clinical practice proposed for the diagnosis of Alzheimer's disease at the Alzheimer's Association of the National Institute of Aging [5, 6], 14 (23.7%) – meet to criteria probable vascular dementia (VD) according to the NINDS-AIREN [7]. 30 patients with mild NCD was divide to amnestic MCI (aMCI) – 9 (15.25%) patients if they had impairment in the memory domein and nonamnestic MCI (naMCI) – 21 (35.59%) if they had impairment in any 1 or more of the nonmemory cognitive domain. There is no patients with early-onset dementia or MCI or family history of AD.

Inclusion criteria were: the objective confirmation of cognitive impairment according to clinical and neuropsychological tests based on criteria of propable AD, probable VD and MCI, presence signs of cerebrovascular and neurodegenerative brain damage according to clinical and neuroimaging methods. Exclusion criteria were: severe somatic diseases, other mental disorders, traumatic brain damage and brain tumors, infections, epilepsy, Parkinson's disease, demyelinating and inherited degenerative diseases, alcohol consumption, intake of drugs that reduce

Table I. Comparison of characteristics between patients with AD, VD and MCI

characteristics	Major NCD (n=29)	Mild NCD (MCI) (n=30)	p value	AD (n=15)	VD (n=14)	p value
Mean age (y)	67.5±0.6	65.6± 0.8	0.0638	67.9±0.8	67.0±0.3	0.3145
Male/female	14/15	18/12	0.1640	5/10	9/5	0.0960
Arterial hypertension	20	17	0.3290	6	14	<0.0001
Smoking	19	11	0.0270	5	14	<0.0001
Diabetes mellitus	13	2	0.0001	3	10	0.0050
Ischemic heart disease	22	5	<0.0001	8	14	0.0030
Acute ischemic events in anamnesis	14	5	0.0090	0	14	<0.0001
Mean MMSE score	20.2±1.64	25.2±0.85	0.0083	18.8±0.56	21.7±0.69	0.0028
Mean MoCA test	18.1±1.67	24.2±0.86	0.0018	16.6±0.50	19.7±0.61	0.0005
Mean FAB test	11.7±0.77	14.3±0,9	0.0327	12.4±0.50	11.1±0.36	0.0468
Mean HIS score	5.6±1.58	–	–	2.4±0.50	9.1±0.77	<0.0001

cognitive function, taking corticosteroids, severe post-stroke deficite, inability to have sufficient verbal contact.

For record vascular risk factors the patient's medical history and medication use was obtain. Hypertension was define by casual blood pressure ≥140/90 mmHg or current use of antihypertensive drugs, diabetes was define by fasting glucose ≥ 7 mmol/l or use glucose-lowering agents.

The control group consisted 30 subjects (mean age 65.7 ± 0.9) without cognitive deficit and serious illnesses. No significant differences observed for age, gender, education level between patients groups and control subjects.

All patients were examine to a comprehensive neuropsychological examination using the following tests and scales: Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Frontal Assessment Battery (FAB), Hachinski's Ischemic Scale – (HIS). The severity of cognitive impairment determined by the Clinical Dementia Rating (CDR). In addition, all patients evaluated using Magnetic Resonance Imaging (MRI).

Serum levels of cytokines of IL-17 and IL-23 was assayed using sandwich ELISA on "Chem Well 2900" immunoanalyzer (Awareness Technology, USA). Test systems using Bender Medsystems, Australia (IL-17 and IL-23) were assay in according with the manufactures instructions.

The work performed in accordance with the principles of the World Health Association Helsinki Declaration "Ethical Principles of Medical Research with Human Involvement as Object of Study" Order of the Ministry of Health of Ukraine No. 690 (dated September 23, 2009). Before inclusion in the study, patients and their relatives were inform with the study protocol and signed voluntary informed consent.

The IBM Statistical Package was use to perform statistical analyses. The level of significance was defined as $p<0.05$. χ^2 test were conducted to compare clinical characteristics and Kruskal-Wallis test was applied to compare the concentration of the IL between different group.

RESULTS

In our study the vascular risk factors that associated with cognitive impairment was higher in patients with major NCD compared with mild NCD. However, in group with VD the incidence of arterial hypertension, smoking, congestive heart failure, diabetes mellitus and anamnesis of acute ischemic events was significantly higher compared with patients with AD (table 1).

The mean scores of MMSE and MoCA test was significantly lower in patients with AD comparable with VD (p=0.0028; p=0.0005), particular in subtest orientation (3.4±0.51 vs 4.8±0.34, p=0.0226), delayed recall (1.8±0.4 vs 2.9±0.2, p=0.0108). Mean HIS score was higher in VD patients.

The detectable serum levels of IL-17 and IL-23 in patients with major NCD, AD and VD presented in table 2.

Levels of detectable interleukins were significantly higher in patients with AD comparable with VD (P=0.0481). IL-17 level was in 10 time higher in AD patients comparable control (p=0.0023). In patients with VD no significant differences with control (p=0.4154), but individual values in patients with VD was significantly greater than normal.

IL-23 level also was significant higher in AD patients than in control group (p=0.0170) and significant differences observed between patients with AD and VD (p=0.0027). Level of IL-23 was in 42 time higher comparable with control and in 12.5 time higher comparable with VD patients. This result confirm that elevate concentration of IL-17 and IL-23 high specific for AD.

When comparison the IL-17 and IL-23 concentration in patients with total mild NCD and control no significant differences were found (p=0.1215; p=0.4733) (table 3). However when compared patients with aMCI and naMCI significant differences found in IL-17 between aMCI and control (p=0.0436).

No significant differences in serum concentration of IL-23 observed in total mild NCD patients and control, but significant differences was found between aMCI patients

Table II. Serum levels of the IL-17 and IL-23 in patients with AD, VD and control

Interleukin concentration, pg/ml Mean±SD	Total major NCD n=29	AD n=15	VD n=14	Control n=30	P value
IL-17	13.11±5.11	22.44±8.92	3.11±1.35	2.10±0.56	P_1=0.0335 P_2=0.0023 P_3=0.4154 P_4=0.0481
IL-23	35.75±15.2	64.33±22.41	5.14±1.62	1.53±0.20	P_1=0.0265 P_2=0.0170 P_3=0.0002 P_4=0.0027

P_1 – differences between major NCD and control
P_2 – differences between AD and control
P_3 – differences between VD and control
P_4 – differences between AD and VD

Table III. Serum levels of the IL-17 and IL-23 in patients with mild NCD and control

Interleukin concentration, pg/ml Mean±SD	Total mild NCD N=30	aMCI n=9	naMCI n=21	Control n=30	P value
IL-17	4.04±1.10	4.36±0.61	3.90±0.58	2.10±0.56	P_1=0.1215 P_2=0.0436 P_3=0.0344 P_4=0.6411
IL-23	1.84±0.38	2.80±0.17	1.43±0.21	1.53±0.20	P_1=0.4733 P_2=0.0019 P_3=0.7376 P_4=0.0004

P_1 – differences between mild NCD and control
P_2 – differences aMCI and control
P_3 – differences naMCI and control
P_4 – differences between aMCI and naMCI

and control (p=0.0019) and aMCI and naMCI patients groups (p=0.0004). Concentration of IL-23 was significantly higher in patients with aMCI compare with naMCI (p=0.0004). Such differences confirm that aMCI may be early stage of AD and elevation of serum concentration IL-17 and IL-23 in patients may be addition markers of risk progression aMCI in AD.

DISCUSSION

Alzheimer's disease (AD) is a neurodegenerative disorder that is the most common cause of dementia. AD is characterize by two core pathologies, the presence of β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs). A number of investigations initially demonstrated that in addition to Aβ plaques and NFT, the brains of patients with AD exhibited evidence of a sustained inflammatory response [8]. This chronic neuroinflammation is attributed to activated microglia cells and the release of numerous cytokines. Many studies now point to the involvement of neuroinflammation playing a fundamental role in the progression of the neuropathological changes that are observe in AD [9, 10]. Such overproduction of IL-6 leads to chronic neuroinflammation and neurodegeneration [11]. IL-1 is a proinflammatory cytokine that is upregulated early in AD development and are considere crucial for β-amyloid plaque deposition. IL-1β is similarly elevated in both MCI and AD patients compared with controls, suggesting that increased IL-1β production begins early and remains elevated as the disease progresses. Specific IL-1β polymorphisms resulting in higher IL-1β production are linked to increased AD risk [12]. The participant of IL-10 that play anti-inflammatory and neuroprotective role in nervous system also investigated in AD [13]. The role of IL-17 and IL-23 is less elucidate. Research demonstrated that IL-23/T17 axis plays a role in AD-associated neuroinflammation and IL-17 in the production of Th17 [14]. In vitro studies suggest that IL-23 might promote Th17 development, stimulate Th17 expansion and prolong IL-17 production [15]. In previous study are observe the elevation of IL-18, IL-23 and IL-17 levels in Chinese patients with AD and differences between males and females [16]. In this study, we compared serum level IL-17 and IL-23 in patients with clinical diagnosis AD and VD. Our results suggest that in AD patients interleukins significantly increase that reflect increase of inflammatory response, which could contribute to the development of neurodegeneration in AD.

Patients with aMCI are consider to be at high risk for AD [17]. Routine use of biomarkers such cerebrospinal fluid $A\beta_{1-42}$ still obstacle for identify the disease etiology [18] and search new biomarkers for it identify and early therapeutic intervention is important aim. In our study IL-17 and IL-23 was statistically significant higher in aMCI patients comparable with control group.

CONCLUSIONS

IL-17 and IL-23 level significant higher in Alzheimer's disease patients compared with control and vascular dementia. Levels of detectable interleukins was higher in aMCI compared with control and significant differences between aMCI and naMCI groups was demonstrate for IL-23. Future investigation may be elucidate a potential role of this interleukins as additional biomarkers for early predict of progression aMCI in Alzheimer's disease.

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ORIGINAL ARTICLE

ACUTE ISCHEMIC STROKE IN WOMEN: EFFICACY OF THE FREE RADICAL SCAVENGER EDARAVONE

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ABSTRACT

The aim: To investigate the effectiveness of usage of the free radical scavenger Edaravone in the therapy of women with AIS.

Materials and methods: A prospective study was conducted of 48 women with AIS, divided into two groups. Patients in the first group (n = 36) were treated with edaravone 30 mg twice a day intravenously. Neuroprotectors were not used in the control group (n = 12). Clinical-instrumental and neurological examination (Glasgow scale (SCG), FOUR, NIHSS, and neuronspecific enolase (NSE) levels) were performed on all patients.

Results: The mean FOUR score in the 1th group increased from 11.04±0.85 to 15.47±0.63 points against 11.39±0.56 to 13.46±1.49 in the control group ($p<0.05$). The level of NSE in control group patients increased 10-fold (from 9.2 to 96.4 ng/ml, $p<0.01$). Subsequently, there was a rapid decrease in NSE level in 1th group, and in the control group until 10 days of treatment, the level of NSE did not reach the reference values ($p<0.05$).

Conclusions: The introduction of edaravone in women with AIS results in positive results already in the acute period of the disease. The use of edaravon was significantly effective on the FOUR scale and the dynamics of NSE levels.

KEY WORDS: ischemic stroke, stroke in women, neuroprotection, edaravon

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INTRODUCTION

Cardiovascular diseases (myocardial infarction, stroke, heart failure, hypertension, and cardiomyopathy) make up 40% of all causes of deaths in men and about 49% – in women [1]. Although deaths from acute ischemic stroke (AIS) have decreased in the world in recent years, it remains one of the major health problems worldwide. It should be noted that there are significant differences between the course of AIS in men and women: from the risk factors to the features of intensive care.

RISK FACTORS

Risk factors differ between men and women: for example, atrial fibrillation and hypertension are higher in women with stroke, and smoking or excessive alcohol consumption is higher in men [2]. The most significant risk factors for men are alcohol and tobacco usage, history of myocardial infarction and peripheral artery disease [3]. For women, factors associated with an increased risk of ischemic stroke are oral contraceptives, pregnancy, and menopause [4]. It has been observed that some risk factors, such as diabetes or smoking, carry a higher risk for women than for men [2].

Women-specific risk factors should be identified in a timely manner to further reduce mortality. For example, atrial fibrillation is widespread in women, requiring appropriate evaluation and prescribing of anticoagulant therapy [5]. Women with AIS and concomitant diabetes have a higher risk of cardiovascular complications and a 50% higher risk of death than men [6]. This fact requires the active detection of carbohydrate metabolism disorders in women.

CLINICAL DIFFERENCES

On average, women have a more pronounced degree of neurological impairment (in particular, higher NIHSS scores), higher mortality and disability compared to men. This is often due to the fact that they have AIS at an older age than men [2, 7]. Interesting differences are observed in the clinical picture of the first manifestations of AIS. Women are more likely to have generalized non-specific stroke symptoms. In particular, in AIS, women are more likely to have symptoms such as disorientation, weakness, changes in mental status [8], headache, changes in consciousness [9], urinary incontinence, visual deficits, diplopia, and dysphasia [3]. On the other hand, nystagmus and sensory disorders are more common in men [8]. These differences sometimes affect the diagnosis and decision making of the treatment and transportation of patients. In addition, women are significantly older than men at the onset of AIS and are more often single or living alone, which also increases the time to be examined and hospitalized.

Table 1. The main characteristics of patients in the study groups (M±m)

Показник	Study groups		
	1th group	Control group	p*
Number of patients, n	36	12	-
Age, years, (Me [Q1; Q3])	68.5 [61.75; 74.0]	69.0 [62.5; 73.5]	>0.05
Height, cm	164.0±8.7	163.6±9.3	>0.05
Weight, kg	74.3±10.9	73.9±8.7	>0.05
BMI, kg/m2	27.6±2.9	27.5±3.8	>0.05
NIHSS score on admission, points	13.4±3.1	12.9±3.7	>0.05

Notes: *p – difference of indexes relative to the comparison group; BMI – the body mass index.

EXAMINATION

Women are less likely to receive brain imaging, Doppler ultrasound, echocardiography, and angiography than men of the same age [3].

DIFFERENCES IN TREATMENT

Surgical treatment of AIS, in particular carotene treatments, is quite popular in high-risk patients. However, carotid endarterectomy is performed less in women than in men, even after adjusting for age and comorbidity. This is due to the higher perioperative risk in women [10] and to the fact that carotid diseases are more common in men. In addition, the main method of treatment for AIS is the introduction of recombinant tissue plasminogen activator (rt-PA) and it also less commonly used in women than in men. The main reasons for this are late hospitalization and the presence of more comorbid pathology [4].

Thus, all of the listed features of AIS in women significantly reduce the ability to receive appropriate and timely care, and ultimately impair treatment outcomes. All of this requires finding other ways to help women with AIS. Medicinal neuroprotection is such therapy that reduces damage, prevents neuronal death at the cellular and molecular levels, or promotes brain cell recovery after acute ischemia or reperfusion [11].

One of the most interesting and promising drugs in the group of neuroprotectors is edaravon, a low molecular weight antioxidant agent that interacts deliberately with peroxyl radicals [12]. Free radicals are one of the main causes of ischemia-related vascular disorders in the brain. They cause the peroxidation of unsaturated fatty acids, which are part of the cell membrane lipids, damages them, which leads to impaired brain function. Edaravon inhibits lipid oxidation by absorbing water-soluble peroxyl radicals that initiate chain reactions, and absorbs fat-soluble peroxyl radicals supporting the chain. In the acute stage of AIS, the drug exhibits a protective effect by suppressing the onset and development of ischemic cerebrovascular disorders such as brain swelling, neurological symptoms, slow neuronal death. This is the way in which edaravon inhibits the early and late stages of the ischemic process and prevents reperfusion injury in AIS [12].

Edaravon was developed and implemented by Japanese company Mitsubishi Tanabe Pharma in 2001. Since then, Edaravon has been successfully prescribed in Japan for the treatment of AIS, and for many years it has been included in the Japanese National AIS Guideline (recommendation level B) [13]. To date, the efficacy and safety of edaravon in the treatment of ischemic stroke has been demonstrated in many clinical studies [14, 15, 16]. However, there is insufficient data on the use of edaravone in the cohort of women undergoing AIS. This led to the need for this study.

THE AIM

The aim of this study is to investigate the effectiveness of usage of the free radical scavenger Edaravone in the therapy of women with acute ischemic stroke.

MATERIALS AND METHODS

To achieve the goal of the study, a prospective comprehensive clinical and neurological and laboratory examination was conducted. 48 patients with acute ischemic stroke were examined. The study was conducted at the intensive care unit of the Kyiv Region Clinical Hospital from September 2018 to August 2019. Prior to the start of the study, patients signed an informed consent. The protocol and the study program were approved by the Shupyk NMAPE Ethics Committee.

The criterion for involvement in the study was: acute ischemic stroke, verified by clinical neurological and neuroimaging methods.

The criterions for exclusion from the study were the presence of a patient with severe comorbid pathology: acute myocardial infarction, acute renal, hepatic and respiratory failure, epilepsy, brain tumor, hemorrhagic stroke.

The patients were divided into two groups. To a patients of the first group (n = 36) was prescribed a solution containing 1.5 mg of edaravone in 1 ml solution at a dose of 30 mg of edaravone (1 ampoule) twice daily, in the morning and in the evening, by intravenous infusion for 30 minutes. Prior to administration, the contents of the ampoule was dissolved in 100 ml of 0.9% sodium chloride solution. The drug was in the form of intravenous drip (40-60 drops per minute). In the control group (n = 12 patients) drugs with the claimed neuroprotective effect were not used. The patients of the presented groups did not differ in basic

Table 2. Results of scores on the FOUR scale, points (M±m)

Parameters	Study days					
	1	3	5	7	9	10
The first group (n=36)	11.04±0.85	12.15±0.91	13.29±1.09	13.98±1.38	15.04±1.16	15.47±0.63
Control group (n=12)	11.39±0.56	11.64±1.22	12.37±1.73	12.64±1.82	13.38±1.53	13.46±1.49
Intergroup differences (p)	>0.05	>0.05	>0.05	>0.05	<0.05*	<0.05*

Note: *- the differences are statistically significant (p<0.05).

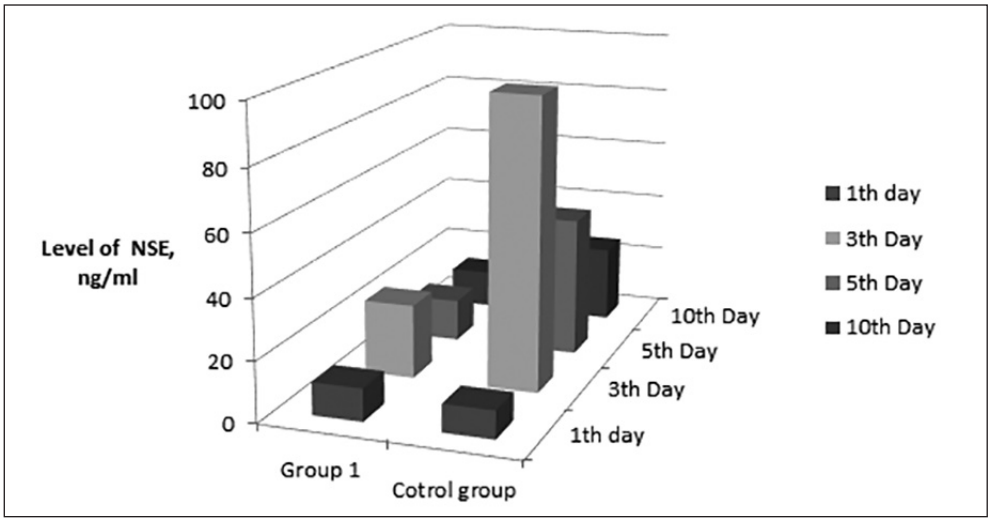

Fig. 1. The dynamics of NSE changes.

anthropometric, anamnestic and clinical-instrumental characteristics, which is shown in Table I.

Examination of patients included clinical and laboratory monitoring of biochemical and coagulatory parameters of blood and urine, comprehensive instrumental examination. Diagnosis of AIS and its localization were verified by computed tomography (CT) and / or magnetic resonance imaging (MRI) of the brain. The following parameters were used as the criteria for the effectiveness of cerebroprotective therapy: assessment of Glasgow Coma Scale (GCS) and FOUR scale, NIHSS score (stroke severity) and Bartel index (daily activity score); the level of neurospecific enolase (NSE; marker of neuronal damage); cerebral oximetry (rSO2; marker of brain oxygenation). These parameters were determined in patients throughout the treatment period, at the end of treatment, a control neuroimaging study (CT or MRI) was performed.

Statistical processing of the results was carried out using statistical analysis packages Microsoft Excel 2010 and SPSS 13.0 using non-parametric methods. The probability of difference between the average quantitative values of the two samples was determined by the Student's t-test. For all parameters measured the average (M), the average error (m). Pearson's χ^2 criterion was used to estimate the likelihood of differences between several relative values.

RESULTS

In the subtype of stroke, patients with large artery atherosclerosis predominated in both groups (group 1 - 25 patients (69.4%), control group - 8 patients (66.7%), p> 0.05). Lacunar strokes were diagnosed: group 1 in 6 patients (16.7%), control group - in 3 patients (25.0%), p> 0.05. Cardioembolic strokes were diagnosed: 1th group – 5 patients (13.9%), control group – 1 patient (8.33%), p> 0.05.

Analysis of GCS estimates showed the presence of positive dynamics in the majority of patients in both groups, with no significant statistical difference (p> 0.05) between the groups. Thus, the mean GCS score in the main group increased from 11.84 ± 2.62 to 13.87 ± 0.94 points against 11.69 ± 3.15 to 13.31 ± 1.78 in the control group (p> 0,05). However, not all women were diagnosed by the Glasgow coma scale (presence of an intubation tube, motor aphasia), so patients' consciousness was evaluated by the FOUR scale (Table II). In the group where the free radical scavenger edaravon was used (1th group), the level of consciousness recovered more rapidly (since the 5th day) than in the control group. According to the analysis of the FOUR scale, within 9-10 days of treatment, the difference between the scores became significant and reached the level of statistical significance: in the edaravon group - 15.47 ± 0.63 points, in the control group - 13.46 ± 1.19 points (p <0.05).

In the analysis of the dynamics of the assessment of stroke severity on the NIHSS scale it was found that patients had predominantly AIS moderate severity (within 10-12 points on the NIHSS), during treatment the number of points gradually decreased without statistical difference between the main and control groups of the study (p> 0.05).

Analyzing the dynamics of NSE changes, the following results were obtained. On the 3rd day of treatment in both groups, the level of NSE exceeded the normal values, and most of all - in patients of the control group the level of NSE

increased 10 times (from 9.2 to 96.4 ng / ml). In group 1, increas in NSE was also observed, but with a much lesser extent (from 10.8 to 24.6 ng / ml, p <0.05 between groups). Subsequently, there was a rapid decrease of the level of NSE, which in the first group reached normal levels by the 10th day of treatment. In the control group, the dynamics of NSE decrease were not so pronounced and by 10 days of treatment the NSE level did not reach the reference values (Fig. 1).

When evaluating the indicators of cerebral oximetry (rSO2), it was found that in patients of different age groups and comorbid conditions, this indicator can vary significantly - from 65 to 90%, and in the case of AIS, the indicator rSO2 over the affected hemisphere. In the course of the study it was noted that patients of the control group experienced a sharp decrease in rSO2 (especially for 3-4 days), followed by a long and slow increase. In patients treated with edaravone, a decrease in rSO2 was less pronounced and less durable, although the difference between study groups was not statistically significant (p> 0.05).

DISCUSSION

As noted, the efficacy of edaravone in the treatment of AIS has been shown in many studies. Thus, according to a Phase III study, when using edaravone in patients with acute ischemic stroke, it was found that in the assessment of functional status on the Rankin scale 3 months after stroke development, a complete absence of functional disorders was noted in 27 of 125 patients (21.6%) against 12 of 125 (9.6%) in patients in the placebo group [17]. It has been reported that, with concomitant use of edaravone with endovascular therapy, better neurological results were observed at hospital discharge [18].

At the same time, information on the efficacy of edaravone mainly relates to long-term treatment outcomes [19]. And our study reflects the effects of edaravone which are developed during the acute period of AIS – the first days and hours of the onset of symptoms.

The dynamics of neurological status assessments that was observed in our study indicate the efficacy of edaravone in the acute period of AIS. Positive treatment outcomes were achieved in all patient groups. However, women receiving edaravone had a more pronounced improvement in neurological symptoms than women of the control group. However, a statistically significant difference was observed only in the dynamics of NSE levels and FOUR scores. We believe that the validity of the positive effect of edaravon on major neurological outcomes in stroke will be fully validated in further randomized trials.

CONCLUSIONS

1. The usage of edaravone in women with AIS leads to positive results already in the acute period of the disease.
2. In particular, the usage of edaravone was significantly effective in terms of neurological status (level of consciousness on the FOUR scale) and the dynamics of levels of neurological markers (NSE).
3. Further studies are needed to clarify the role and location of edaravone in the ischemic stroke intensive care setting.

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ORIGINAL ARTICLE

THE ROLE OF ZONULIN IN THE DEVELOPMENT OF LIVER FIBROSIS IN OBESE ADOLESCENTS

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ABSTRACT

The aim: To study the relationship between zonulin level and PNFI (pediatric non-alcoholic fatty liver disease fibrosis index) in obese adolescents.

Material and methods: A total of fifty-nine obese subjects aged 12-17 years and thirteen healthy subjects were included in the study. Clinical, biochemical parameters, including serum zonulin, were examined and abdominal ultrasound examination was performed. For the assessment of liver fibrosis PNFI was calculated.

Results: According to ultrasound examination 71,2% of obese adolescents had non-alcoholic fatty liver disease (NAFLD). Calculation of PNFI showed that 25,4% of obese subjects had fibrotic processes in the liver. Obese adolescents had significantly higher zonulin levels compared to normal weight peers – 91,8±3,1 vs 15,9±5,1 respectively ($p<0,01$). A significant positive correlation was established between zonulin levels and such parameters as body mass index, waist circumference / height ratio, triglycerides, very low-density lipoprotein cholesterol, insulin, homeostasis model assessment of insulin resistance, PNFI ($p<0,05$).

Conclusions: The level of zonulin increases with an increase of the index for evaluating liver fibrosis (PNFI) in obese adolescents. This may indicate the effect of the state of the intestinal barrier on the development and progression of obesity-related liver pathology, namely NAFLD, in obese adolescents.

KEY WORDS: obesity, NAFLD, zonulin, adolescents

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INTRODUCTION

THE PREVALENCE OF OBESITY AND NAFLD

Today, obesity is becoming one of the most common non-communicable diseases in children. According to WHO, over 340 million adolescents and children between the ages of 5 and 19 and about 41 million children under 5 years of age are overweight or obese [1]. Obesity, type 2 diabetes mellitus, hypertension, atherogenic dyslipidemia, and nonalcoholic fatty liver disease (NAFLD) are linked by a common pathogenesis and are components of the metabolic syndrome [2]. Pathological changes in NAFLD are characterized by a spectrum of conditions associated with the presence of vesicular steatosis of the liver. It may eventually progress to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis, which with high probability leads to the development of hepatocellular carcinoma in adults [3]. The prevalence of NAFLD in children and adolescents is growing in parallel with the obesity epidemic in the world [4]. This is confirmed by data from a recent meta-analysis, which revealed that the prevalence of NAFLD in children and adolescents was 7,6% in the general population and 34,2% in obese patients [5].

Recent data indicate that among all characteristic histological changes in the liver in NAFLD, fibrosis has the highest prognostic value for the progression of the disease and the development of complications [6]. The «gold standard» for assessing liver fibrosis is a biopsy, however, due to the fact that the procedure is technically demanding and involves the risk of complications, the use of non-invasive methods for assessing liver fibrosis in obese children is a topical issue in pediatrics. Today, a number of non-invasive tests have been developed to assess fibrotic changes in the liver, which are already used in pediatric practice [7]. The PNFI (pediatric NAFLD fibrosis index) developed specifically for evaluating liver fibrosis in childhood is of particular interest [8].

THE RELATIONSHIP BETWEEN INTESTINAL BARRIER INTEGRITY, OBESITY AND NAFLD

Qualitative and quantitative changes in the diet of obese children and adolescents, namely, the use of food rich in fats and easily digestible carbohydrates, decreased intake of fiber, etc., significantly affect the intestinal biocenosis and lead to changes in the composition of the intestinal microbiota [9]. In recent years, there has been an increasing number of studies demonstrating a close relationship between the intestinal microbiota and the liver. This natural connection is called the «gut-liver axis» [10]. New data on the relationship between the intestines and liver provide a basis for revising the understanding of the pathogenesis of a such obesity-associated pathology as NAFLD. Based on recent studies a «multiple parallel hits» hypothesis has

been proposed, according to which the pathogenesis of NAFLD involves the influence of various factors on the liver tissue, including the gut microbiota and the products of its interaction with the host organism against the background of oxidative stress [11].

Dysbiosis is considered a trigger for increased synthesis of zonulin, a protein that is one of the regulators of intestinal permeability by modulating intercellular tight junctions. Human zonulin is a recently discovered 47-kDa protein that is structurally similar to Vibrio cholerae's zonula occludens toxin [12]. Zonulin is thought to activate the epidermal growth factor receptor (EGFR) through direct binding and / or through a protease-activated receptor (PAR2). This leads to the expansion of intercellular tight junctions in the intestinal wall and, as a result, to increased permeability of the intestinal barrier (the so-called «leaky gut syndrome») [13]. Many components of microbial cells, including the lipopolysaccharide of gram-negative bacteria, flagelin, linoleic acid, and peptidoglycans are so-called pathogen-associated molecular patterns that are recognized by specific Toll-and Nod-like receptors. Under normal conditions, a small amount of bacterial products from the intestine enters the liver through the portal circulation, where they are eliminated by Kupfer cells [14]. Increased permeability of the intestinal barrier leads to an excessive flow of bacteria and their products to the bloodstream and liver, and thus, through stimulation of Toll - and Nod-like receptors, causes activation of signalling cascades and the release of a large number of cytokines and chemokines. This triggers the process of chronic low-grade inflammation, which gives impulse to the progression of obesity and its complications [15].

Recently published studies have shown the highest levels of circulating zonulin in obese adults compared to healthy ones [15]. The results of intestinal permeability studies confirm the role of intestinal barrier dysfunction in the development and progression of not only obesity but also associated pathology. Miele et al were among the first to provide evidence that NAFLD in adult patients is associated with increased intestinal permeability which is caused by violation of intercellular tight junctions [16]. Current research shows that changes in intestinal permeability in obesity and NAFLD are already present in children and adolescents as well. Küme T et al in their work showed that obese children had significantly higher zonulin levels than their healthy peers [17]. Similar data were obtained in the study by Kim I. N. et al. [18]. Zonulin levels were significantly higher in obese children with NAFLD in the work by L. Pacifico et al. [19]. The levels of this protein was also higher in children with NAFLD compared to the control group in studies conducted in Turkey [20] and Italy [21].

These data suggest the possibility of a link between the composition of the gut microbiota, violation of the intestinal barrier and increased levels of endotoxemia, which leads to *chronic* low-grade *inflammation* and contributes to the development of obesity and its complications [10, 11, 14]. However, there are fewer such studies in children and adolescents than in adults, making the question of the impact of disturbed intestinal permeability in the pathogenesis of obesity and NAFLD relevant.

THE AIM

To study the relationship between zonulin level and PNFI in obese adolescents.

MATERIALS AND METHODS

STUDY SUBJECTS

The study involved fifty-nine obese adolescents aged 12-17 years (45,8% - boys and 54,2% - girls) who were treated at the SI «Institute for Children and Adolescent Health Care at the National Academy of Medical Sciences of Ukraine» and 13 healthy children with normal body mass index (BMI) of similar age and gender. The diagnosis of obesity was established according to the ICD-10. NAFLD was diagnosed on the basis of an abdominal ultrasound examination. Other causes of liver disease, including viral infections (cytomegalovirus and Epstein-Barr virus, hepatitis A, E and G), autoimmune hepatitis, Wilson's disease, cystic fibrosis, celiac disease, etc. were excluded in all patients.

Anthropometric examination included measurement of standing height, body weight, waist circumference (WC), hip circumference (HC), calculation of the WC/HC ratio, the WC/height ratio (indicators of abdominal obesity), body mass index (BMI) using the formula: BMI = body weight (kg) / height (m^2). The BMI was assessed according to the standards of WHO. The subjects were treated according to the Declaration of Helsinki and the study was approved by the Ethics Committee of the Kharkiv Medical Academy of Postgraduate Education (No 5, November 2019), Kharkiv, Ukraine. All subjects gave their written informed consent before entering the study.

LABORATORY AND INSTRUMENTAL METHODS OF RESEARCH

The venous blood samples were collected after 10-12 hours of night fasting. The concentrations of serum glucose, immunoreactive insulin (IRI), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamaglutamyltranspeptidase (GGTP), total bilirubin, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) were measured. The kits which were used in the study were EKF-diagnostic GmbH (glucose), DRG (IRI), Spainlab (ALT, AST, total bilirubin, TC, TG, HDL-C, GGTP) according to the manufacturer's instructions. The concentrations of low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated using Friedewald formula: LDL-C (mmol/l) = TC (mmol/l) - HDL-C (mmol/l) – TG (mmol/l)/2,2; VLDL-C (mmol/l) = TG (mmol/l)/2,2 [22]. Insulin resistance was assessed by calculating the homeostasis model assessment of insulin resistance (HOMA-IR): HOMA-IR = fasting glucose (mmol/l) × fasting IRI (μIU/ml)/22,5 [23]. Part of the col-

Table I. The characteristic of clinical and laboratory parameters of the examined adolescents (mean ± SE).

Parameters	Obese subjects (n=59)	Control subjects (n=13)
Age (years)	14,53±0,21	14,46±0,38
Height (cm)	168,06±1,24	168,69±1,97
Weight (kg)	87,41±2,03	54,07±2,14**
BMI (kg/m2)	30,82±0,49	18,98±0,45**
WC (cm)	93,13±1,27	65,54±1,08**
HC (cm)	111,98±0,97	89,61±1,72**
WC / HC (cm)	0,83±0,01	0,72±0,01**
WC / Height (cm)	0,56±0,01	0,34±0,01**
AST (U/L)	26,52±1,41	25,23±1,33
ALT (U/L)	27,26±1,56	21,53±1,54*
GGTP (U/L)	23,13±1,04	16,34±1,16**
Total bilirubin (μmol/l)	16,55±0,81	13,74±0,93*
TC (mmol/l)	5,17±0,11	4,23±0,21**
TG (mmol/l)	1,45±0,08	0,85±0,09**
LDL-C (mmol/l)	2,89±0,11	2,26±0,17**
VLDL-C (mmol/l)	0,65±0,03	0,38±0,08**
HDL-C (mmol/l)	1,61±0,05	1,64±0,19
Glucose (mmol/l)	4,77±0,06	4,34±0,09**
IRI (μIU/ml)	24,71±1,78	11,52±0,71**
HOMA-IR	5,26±0,37	2,21±0,12**
Zonulin (ng/ml)	91,89±3,12	15,96±5,1**

Note: BMI – body mass index, WC – waist circumference, HC – hip circumference, WC/HC – the ratio of waist circumference to hip circumference, WC/height - the ratio of waist circumference to height, AST – aspartate aminotransferase, ALT - alanine aminotransferase, GGTP - gamaglutamyltranspeptidase, TC - total cholesterol, TG - triglycerides, VLDL-C - very low-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C - high-density lipoprotein cholesterol, IRI - immunoreactive insulin, HOMA-IR - homeostasis model assessment of insulin resistance.
* - p<0,05, ** - p<0,01.

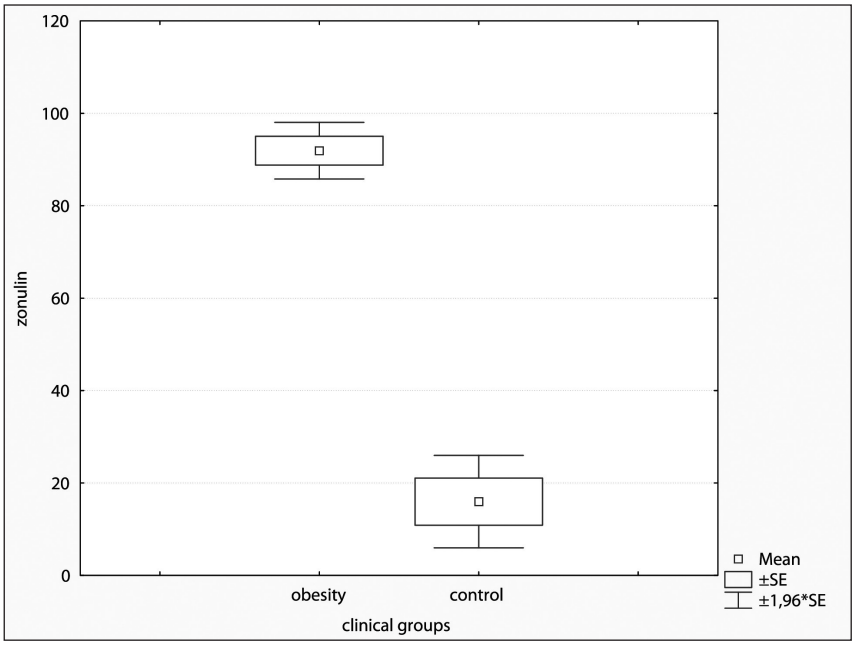

Fig.1. Zonulin levels in obese and healthy subjects

lected blood was centrifuged and stored at -20°C for further analysis of zonulin levels by using an ELISA kit (Elabscience) according to the manufacturer's instructions. Ultrasound examination was performed on an empty stomach using an «ULTIMA SM-50» with a 3,5-5 MHz convex transducer and a 7,5-10 MHz linear transducer.

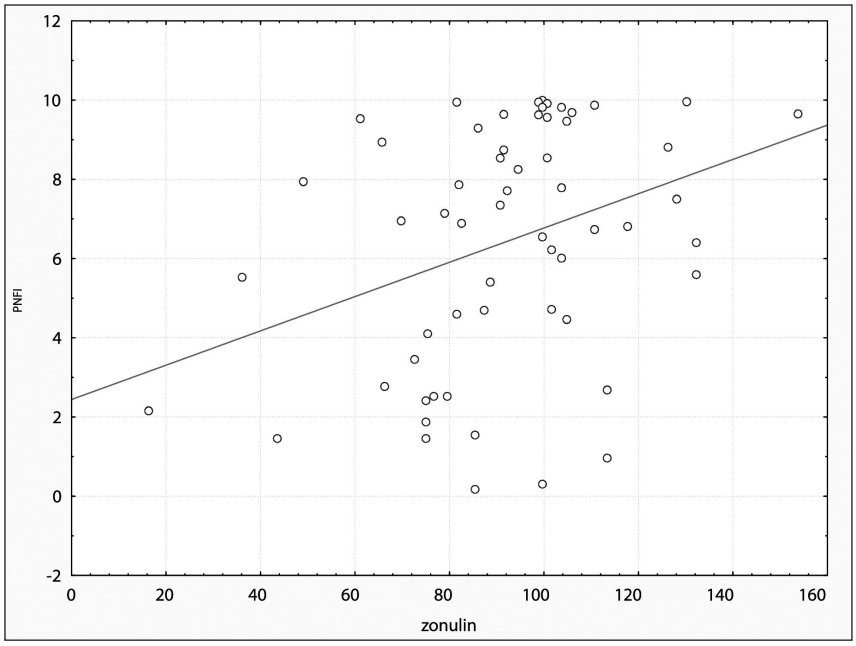

Fig. 2. Association between zonulin and PNFI in obese adolescents

CALCULATION OF PNFI

The PNFI (pediatric NAFLD fibrosis index) was calculated on the basis of TG levels, age and WC: PNFI = $1 / (1+e^{-lp}) \times 10$, where lp = $6{,}539 \times \log_e$ [age (years)] + $0{,}207 \times$ WC (cm) + $1{,}957 \times \log_e$ [TG (mg/dl)] – 10,074. According to the method, the value of the index ≥ 9 indicates the presence of fibrotic changes in the liver of the patient [8].

STATISTICAL ANALYSIS

Statistical analyses were performed by *Statistica 6*.0. The data were expressed as mean ± SE. The distribution of the data was evaluated with the Kolmogorov-Smirnov test. Differences between groups were tested using Student's t-test for the variables conforming to the Gaussian distribution and Mann–Whitney U test for non-Gaussian variables. The data was analyzed by linear correlation. A p-value < 0,05 was considered statistically significant.

RESULTS AND DISCUSSION

The study revealed that 79,7% of patients had increased appetite, 52,5% - abdominal pain, 40,7% - belching, 37,2% - heartburn, 30,5% - nausea, 28,8% - flatulence, 23,7% - constipation, and 15,2% had loose stools. Objective examination of patients revealed pain in the right hypochondrium in 40,6% of adolescents, in the epigastrium in 33,8%, liver enlargement was noted in 79,6%. The characteristic of clinical and laboratory parameters of the examined adolescents is presented in Table I.

As expected among the anthropometric parameters significantly higher in obese adolescents were weight, BMI, WC, HC, WC/HC ratio, WC/ height. There was no significant difference in age and height between the groups. Among the laboratory parameters the levels of IRI, glucose, HOMA-IR, total bilirubin, total cholesterol, TG, LDL-C, VLDL-C, GGTP and zonulin were significantly higher in obese adolescents (Figure 1). It should be menshioned that high GGTP levels are associated with fibrotic changes in the liver, which was noted in the report of the ESPGHAN Hepatology Committee [24]. The AST levels did not differ significantly between the groups. The mean ALT level was higher in obese adolescents, although it remained within the age range. The results obtained in our study confirm the opinion that children with obesity and NAFLD even with normal or slightly elevated ALT levels may already have significant histological abnormalities, including fibrosis [24, 25].

NAFLD was diagnosed in 71,2% of obese adolescents according to ultrasound examination. The calculation of PNFI showed the presence of fibrotic changes in a liver in 25,4% of obese adolescents. Correlation analysis revealed a significant and positive link between zonulin levels and such indicators as BMI (r = 0,37; p = 0,009), WC/height (r = 0,39; p = 0,002), TG (r = 0,28; p = 0,039), VLDL-C (r = 0,28; p = 0,039), IRI (r = 0,26; p = 0,045), HOMA-IR (r = 0,27; p = 0,042), PNFI (r = 0,34; p = 0,008) (Figure 2).

The present study revealed a significantly higher serum zonulin levels in obese adolescents compared to healthy peers. Our findings are consistent with previous studies in both adults [15] and children, adolescents [17, 18, 19, 20, 21].

There were a significant positive correlation links between zonulin levels and levels of TG, VLDL-C, IRI, HOMA-IR, which demonstrate the association between the violation of lipid and carbohydrate metabolism and increased intestinal permeability in obesity. There was also a significant positive correlation between zonulin levels and such anthropometric parameters as BMI, WC / height ratio. This confirms the destructive effect of obesity, especially abdominal type of it, on the state of the intestinal barrier and the development of comorbid pathology. We found a significant positive correlation between zonulin levels

and the index for assessing liver fibrosis PNFI, which may indicate the influence of increased intestinal permeability on the development of NAFLD in obese adolescents.

CONCLUSIONS

1. NAFLD was diagnosed in 2/3 of obese adolescents, and in 1/4 the presence of fibrotic changes of a liver according to the PNFI was noted.
2. A significantly higher serum zonulin levels were observed in obese adolescents compared to healthy peers.
3. The level of zonulin increases in parallel with the PNFI in obese adolescents. This fact may be an evidence of the influence of intestinal permeability on the development and progression of NAFLD. A significant positive correlation between zonulin levels and such parameters as BMI, WC / height, TG, VLDL-C, insulin, HOMA-IR was noted.
4. An in-depth understanding of the causal link underlying the identified data is needed. This will lead to improved diagnosis of NAFLD at an early stage and enable the development of new therapeutic approaches aimed at regulating the gut microbiota in obese children and adolescents.

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ORIGINAL ARTICLE

ONE HEALTH: ANTIBIOTIC-RESISTANT BACTERIA CONTAMINATION IN FRESH VEGETABLES SOLD AT A RETAIL MARKETS IN KYIV, UKRAINE

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ABSTRACT

The aim: To obtain the first estimates data on the occurrence of antibiotic-resistant bacteria in a wide range of fresh vegetables available in the Kyiv city markets.

Materials and methods: We performed a multicenter study. Fresh vegetables samples were collected of the six different commodity groups from eleven of retail stores located in Kyiv, Ukraine. Samples were tested for up to eight bacteria of concern. The susceptibility to antibiotics was determined by disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing.

Results: The antibiotic-resistant bacteria contamination in the fresh vegetables was 24.3%. The contamination among organic produce was significantly higher than in conventionally products. Contamination was found to be higher in leafy vegetables. The predominant contaminated bacteria were: *Escherichia coli, Enterobacter* spp. and *Enterococcus faecalis*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, E. faecium*, *Staphylococcus aureus* and *Acinetobacter* spp. The overall proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriaceae was 36.8% and of methicillin-resistance in *S. aureus* (MRSA) 10.7%. The prevalence of ESBL production among E. coli isolates was significantly higher than in *K. pneumoniae.* Vancomycin resistance was observed in 3.1% of isolated enterococci (VRE). Carbapenem resistance was identified in 35.3% of *P.aeruginosa* isolates and 66.8% of *Acinetibacter* spp. isolates. Resistance to third-generation cephalosporins was observed in 9.7% *K. pneumoniae* and *E.coli* in 14.2% isolates.

Conclusions: Research has shown that the majority of fresh vegetables available in Kiev markets is contaminated with antibiotic-resistant bacteria and is a potential vehicle for the transmission of these pathogens to consumers.

KEY WORDS: Fresh Vegetables, Bacterial contamination, Retail, Antimicrobial Resistance, Ukraine

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INTRODUCTION

Fresh vegetables are well recognized as important parts of a nutritious and healthy diet of human worldwide. Therefore, consumers demand the variety and availability of these products all year round. This has impacted international trade in Ukraine, where many fresh fruits and vegetables are imported. Currently, Ukraine is a importer of fresh fruits and vegetables year round (leafy greens, soft fruits, citrus fruits, grapes, cauliflower, broccoli, onions, bananas, beans and carrots, peppers, tomatoes, avocados, cucumbers and asparagus).

In recent years, internationally, there has been an increasing the number of outbreaks of fresh produce-associated foodborne illness and efforts are being made to resolve these problems [1-5]. Are more frequently linked to these outbreaks; for example, leafy greens, such as lettuce [6] and spinach [7, 8], and fresh herbs, such as parsley and basil [9, 10], green onions [6], tomatoes [11], watermelon [12], are well-recognized potential sources of bacterial infections [4, 5, 7, 9]. As with other countries, Ukraine has seen an increased number of foodborne illness outbreaks linked to fresh produce in the last decades. However, there are no published data on human outbreaks associated with fresh vegetables in Ukraine.

The potential for transfer of antibiotic-resistant bacteria from fresh vegetables into the human population is cause for concern. The success of treating infectious patients, including in foodborne outbreaks, depends on many factors, including resistance to infectious agents due to antimicrobial activity. Optimally, a given antibiotic should be selected based on the safety profile and local drug susceptibility. However, in Ukraine, there is no targeted surveillance for emerging microbiological hazards including antibiotic-resistance bacteria contamination in fresh vegetables at the retail level. The previous reports of antimicrobial resistance in Ukraine have been limited only to healthcare-associated infections [13, 14].

THE AIM

The aim of this study was to obtain the first estimates data on the occurrence of antibiotic-resistant bacteria in a wide range of fresh vegetables available in the Kyiv city markets.

MATERIALS AND METHODS

STUDY DESIGN AND SAMPLING COLLECTION

We performed a multicenter study. Samples were collected between January 1st, 2017 and December 31st, 2019 for each of the six fresh vegetables commodity groups (leafy vegetables, leafy herbs, green onions, cucumbers, berries and tomatoes) from eleven of retail stores located in Kyiv, Ukraine.

The retail stores were selected based on geographic and demographic considerations and the corresponding number of samples was collected in proportion to the relative population of the respective areas of Kyiv city, Ukraine. The goal of this sampling approach was to obtain a large set of samples that would be representative of the targeted food commodities available to Ukrainians in Kyiv city at retail during the time of the study (Table I).

The number of fresh vegetables samples collected in each season was impacted by the availability of the targeted commodity in the Ukrainian market at the time of sampling. Domestic samples were collected during the summer months and imported samples were collected primarily in the fall, winter and spring months. The target sample population consisted of all units of the targeted commodity available at retail to consumers as per the sampling design.

MICROBIOLOGICAL METHODS

In this study samples from fresh vegetables were analysed for the presence of up to eight bacterial species (*Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Enterobacter* spp., *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetibacter* spp.) using the methods published in Ministry of Health of Ukraine Compendium of Analytical Methods for the Microbiological Analysis of Foods. These methods have been fully validated for the analysis of fresh fruits and vegetables and are used for the regulatory testing of foods and in food safety investigations. The analyses of bacterial pathogens were performed using enrichment methods, confirmed by isolation, purification and identification procedures. These analyses were done on 25 g of sample. Samples where bacterial pathogens were detected and confirmed in 25 g of products, or where generic *E. coli* levels were found to be >100 CFU or MPN/g, were reported as positive. Microbial isolates were identified using standard microbiological techniques, including automated microbiology testing (Vitek-2; bioMe´rieux, Marcy l'Etoile, France), and antibiotic susceptibility testing was performed by using the disk diffusion method (Kirby – Bauer antibiotic testing). Isolates were categorized as susceptible or resistant by Clinical and Laboratory Standards Institute criteria (CLSI, 2013). Strains in the intermediate range were classified as resistant for data analysis.

ETHICS

The study was approved by the Ethics Committee of Shupyk National Medical Academy of Postgraduate Education (Kyiv, Ukraine).

STATISTICAL ANALYSIS

A statistical methodology was developed to investigate the presence of the seven bacterial species in the chosen fresh vegetables over the three years of sampling. The analyses are based on the percentage of positive samples for a given bacterial species and for all bacterial species combined (i.e., global sample result). The analysis of statistical data was performed using Excel (Microsoft Corp., Redmond, WA, USA). Results are expressed as median (range), mean standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. The estimated prevalence is defined as the overall percentage of positive samples – either for a given bacterium or for all bacteria studied (global sample result). The Wilson confidence interval (i.e., a two-sided 95% confidence interval for the proportion) was used to estimate the prevalence of bacteria. Statistical significance was defined as $P < 0.05$.

RESULTS

ANTIBIOTIC-RESISTANT BACTERIA CONTAMINATION

A total 13,694 of fresh vegetables samples for six different commodity groups were collected and tested for the presence of a variety of bacteria over a three year period (January 1st, 2017 and December 31st, 2019). The prevalence of the antibiotic-resistant bacteria contamination in fresh vegetables was 24.3% (3,326/13,694) [95% CI 24%, 24.6%, p <0.0001].

The contaminated fresh vegetables samples consisted of imported and domestic products (3.1% and 21.2%, respectively), including a conventionally and organically grown products (5.1% and 19.2%). The prevalence of the bacterial contamination were: leafy vegetables - 34.6% [95% CI 33.6%, 35.6%, p <0.0001], leafy herbs - 40.4% [95% CI 39.5%, 41.3%, p <0.0001], tomatoes - 8.6% [95% CI 8.0%, 9.2%, p <0.0001], green onions - 15.9% [95% CI 15.1%, 16.7%, p <0.0001], cucumbers - 11.6% [95% CI 10.9%, 12.3%, p <0.0001] and berries - 30.7% [95% CI 29.7%, 31.7%, p <0.0001]. Positive rates and prevalence of bacterial contamination in all commodities are summarized in Table II.

The predominant contaminated fresh vegetables were: strawberries (73%, 95% CI 71.1%, 74.9%), parsley (66.1%, 95% CI 64.3%, 67.9%), dill (42.3%, 95% CI 40.0%, 44.4%), cilantro (40.4%, 95% CI 38.3%, 42.5%), leaf lettuce (38.9%, 95% CI 36.7%, 40.9%), spinach (38%, 95% CI 36.0%, 40.0%), basil (35.4%, 95% CI 33.4%, 37.4%) and mixed greens (33.2%, 95% CI 31.2%, 35.2%), followed by head lettuce (26.4%, 95% CI 24.9%, 29.1%) and blueberries (26.1%, 95% CI 24.1%, 28.1%). Low bacterial contamination has been found in tomatoes (8.6%, 95% CI 8.0%, 9.2%), raspberries (10.7%, 95% CI 9.2%, 12.2%) and cucumbers (11.5%, 95% CI 10.9%, 12.3%).

BACTERIAL PATHOGENS IN FRESH VEGETABLES

A total of 8179 strains isolated from 3326 contaminated fresh vegetables. Gram-positive organisms accounted for

Table I. Number of samples by commodity groups

Commodity	Total (n = 13694)	Number of samples			
		Product Origin		Production Type	
		Domestic	Imported	Conventional	Organic
Leafy Vegetables	2132	1594	538	465	1667
Leafy Herbs	2812	2390	422	597	2215
Tomatoes	2332	1007	1325	996	1336
Green Onions	2014	1738	276	131	1883
Cucumbers	2212	955	1257	936	1276
Berries	2192	1499	693	716	1476

Table II. Bacterial contamination in fresh vegetables

Commodity	Number of samples	Number of positive samples			
		Product Origin		Production Type	
		Domestic	Imported	Conventional	Organic
Leafy Vegetables	2132	642 (30.1%)	96 (4.5%)	112 (5.3%)	626 (29.3%)
Leafy Herbs	2812	1012 (35.6%)	124 (4.4%)	167 (5.9%)	969 (34.1%)
Tomatoes	2332	145 (6.2%)	56 (2.4%)	58 (2.5%)	143 (6.1%)
Green Onions	2014	289 (14.3%)	32 (1.6%)	93 (4.6%)	228 (11.3%)
Cucumbers	2212	240 (10.8%)	16 (0.7%)	76 (3.4%)	180 (8.1%)
Berries	2192	579 (26.4%)	95 (4.3%)	189 (8.6%)	485 (22.1%)
Total	13694	2907 (21.2%)	419 (3.1%)	695 (5.1%)	2631 (19.2%)

27% [95% CI 26.5%, 27.5%, p <0.0001] of all strains and gram-negative organisms accounted 73% [95% CI 72.5%, 73.5%, p <0.0001], respectively. Enterobacteriaceae were the most frequently isolated group of organisms from contaminated fresh vegetables (64.3%, 95% CI 63.8%, 64.8%, p <0.0001). The predominant pathogens were: *E. coli* (35.8%, 95% CI 35.3%, 36.3%), *Enterobacter* spp. (17.8%, 95% CI 17.4%, 18.2%) and *E. faecalis* (17.1%, 95% CI 16.7%, 17.5%), followed by *K. pneumoniae* (10.7%, 95% CI 10.4%, 11%), *P. aeruginosa* (6.5%, 95% CI 6.2%, 6.8%), *E. faecium* (6%, 95% CI 5,3%, 6,7%), *S. aureus* (4%, 95% CI 3.8%, 4.2%) and *Acinetobacter* spp.(2.2%, 95% CI 2.0%, 2.4%) (Table III and IV).

ANTIMICROBIAL RESISTANCE

Because most commensally bacteria have natural gene transfer mechanisms and can be resistant to multiple antimicrobials, it is important to characterize the strains that have been isolated from food. Antimicrobial susceptibility tests were performed on a total of 2260 isolates of Gram-positive cocci and 5919 gram-negative organisms. The antimicrobials used in antimicrobial susceptibility testing included those commonly used as therapeutic agents. Varying degrees of resistance to most antimicrobials tested were found.

The staphylococcal isolates displayed a remarkable resistance to penicillin (83.1%) and erythromycin (65.2%), although there were some differences depending on the fresh vegetables. Staphylococcal isolates showed susceptibility to most of the other antimicrobials tested. No strains resistant to linezolid, teicoplanin, vancomycin, tigecycline, and fusidic acid were found. Methicillin-resistance was observed in 10.7% of *S. aureus*. Regarding the genus *Enterococcus, E. faecalis* isolates and *E. faecium* were not sensitive to those antibiotics to which they are intrinsically resistant (cefuroxime, clindamycin, and trimethoprim-sulfamethoxazole) and 85.3% of them were resistant to erythromycin. Approximately, 19% of the *E. faecalis* isolates displayed resistance to high levels of aminoglycosides (gentamycin, tobramycin) and around 7.9% was resistant to quinolones (ciprofloxacin and levofloxacin), and 4% to glycopeptides (vancomycin and teicoplanin). Vancomycin resistance was observwd in 3.1% of isolated enterococci (VRE).

The overall proportion of extended spectrum beta-lactamases (ESBL) production among Enterobacte-

Table III. Distribution of gram-positive organisms in fresh vegetables.

Commodity	Number of samples	Microorganism		
		S. aureus	*E. faecalis*	*E. faecium*
Leafy Vegetables	1826	82 (4.5%)	394 (21.6%)	122 (6.7%)
Leafy Herbs	2634	54 (2.1%)	264 (10%)	103 (3.9%)
Tomatoes	685	97 (14.2%)	164 (23.9%)	43 (6.3%)
Green Onions	1032	42 (4.1%)	122 (11.8%)	69 (6.7%)
Cucumbers	697	72 (10.3%)	186 (26.7%)	39 (5.6%)
Berries	1355	27 (2%)	268 (19.8%)	112 (8.3%)
Total	8179	324 (4%)	1398 (17.1%)	488 (6%)

Table IV. Distribution of gram-negative organisms in fresh vegetables

	Number of samples	Microorganism				
		E.coli	*Enterobacter spp.*	*K.pneumoniae*	*P. aeruginosa*	*Acinetibacter spp.*
Leafy Vegetables	1826	734 (40.2%)	224 (12.3%)	121 (6.6%)	118 (6.5%)	31 (1.7%)
Leafy Herbs	2634	1122 (42.6%)	661 (25.1%)	257 (9.8%)	134 (5.1%)	39 (1.5%)
Tomatoes	685	143 (20.9%)	97 (14.2%)	89 (13%)	29 (4.2%)	23 (3.4%)
Green Onions	1032	297 (28.8%)	211 (20.4%)	137 (13.3%)	121 (11.7%)	33 (3.2%)
Cucumbers	697	168 (24.1%)	83 (11.9%)	94 (13.5%)	31 (4.4%)	24 (3.4%)
Berries	1355	462 (34.1%)	177 (13.1%)	179 (13.2%)	101 (7.5%)	29 (2.1%)
Total	8179	2926 (35.8%)	1453 (17.8%)	877 (10.7%)	534 (6.5%)	179 (2.2%)

riaceae was 36.8%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K. pneumoniae* (33.5%, vs 14.8%, p < 0.001). *E.coli* was most sensitive (>95%) to ertapenem (100%), cefotaxime (99.1%), ceftazidime (99.4%), fosfomycin (98.7%), imipenem (98.9%), piperacillin/tazobactam (97.3%), and gentamycin (94.5%) but least susceptibility (<70%) was observed for moxifloxacin (54.2%), cefuroxime (65.8%), amoxicillin (67.3%), and levofloxacin (68.5%). Resistance to third-generation cephalosporins was observed in 14.2% *E.coli* isolates. No strains of *E.coli* resistant to ertapenem were found. *Enterobacter* spp. isolates ones exhibited a noticeable percentage of resistance against ampicillin/sulbactam (61.9%), amoxicillin/clavulanic acid (60.2%), ciprofloxacin (47.8%), clindamycin (52.2%), ampicillin (52.1%), amikacin (43.8%), and gentamycin (43.5%), cefaperazon (41.3%) and ceftriaxon (34.1%). No strains of *Enterobacter* spp. resistant to cefepime, meropenem, imipenem, and ertapenem were found. *K. pneumoniae* isolates showed susceptibility to most of the other antimicrobials tested, while these isolates ones exhibited a noticeable percentage of resistance against ampicillin (51.5%), amoxicillin/clavulanic acid (41.4%), ofloxacin (33.1%), and ciprofloxacin (29.7%). No strains resistant to piperacillin/tazobactam and ertapenem were found. Resistance to third-generation cephalosporins was observed in 9.7% *K. pneumoniae* isolates. Carbapenem resistance was identified in 35.3% of *P.aeruginosa* isolates and 66.8% of *Acinetibacter* spp. isolates.

In isolates from fresh vegetables, the proportions of ESBL production were higher in domestic products (33.1% vs 3.7%, $p < 0.001$). Compared with organic production, isolates from domestic conventional products were also more frequently resistant to aminoglycosides (23.3%, vs 13.0%, $p= 0.031$) and fluoroquinolones (31.7% vs 8.3%, p < 0.001). All vancomycin-resistant enterococci and carbopenem-resistant *P. aeruginosa* were isolated from domestic conventional products.

DISCUSSION

This is the first study were to obtain of the current prevalence of the occurrence of antibiotic-resistant bacteria contamination in a wide range of fresh vegetables available in the city markets of Ukraine. In this study the antibiotic-resistant bacteria contamination in the fresh vegetables was 24.3%. The contamination among organic produce was significantly higher than in conventionally products. Contamination of leafy vegetables by the bacterial pathogens investigated was found to be frequent

and widespread occurrence. Leafy vegetables appeared to be more frequently contaminated in the summer. This trend extended to both imported and domestic leafy vegetables, and was particularly strong in organic produce. Several studies on this subject [15-17] found that organic produce was more susceptible to bacterial contamination. Our findings support this observation and indicate that summertime is the prime period to observe this difference in hygienic quality in organic leafy vegetables.

Results of this study suggest that fresh vegetables commonly purchased from grocery stores in Ukraine are a source for bacterial species and a public health concern. All eight bacteria studied belong to the group of pathogens that cause the majority of nosocomial infections. The predominant contamination of antibiotic-resistant bacteria were: *E. coli*, *Enterobacter* spp. and *E. faecalis*, followed by *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *Acinetobacter* spp. *S.aureus* were cultured most often from tomatoes (14.2%) and cucumbers (10.3%), *E.faecalis* from cucumbers (26.7%) and tomatoes (23.9%), *E.faecium* from berries (8.3%), green onions (6.7%) and leafy vegetables (6.7%), *E.coli* from leafy herbs (42.6%), leafy vegetables (40.2%) and berries (34.1%), *Enterobacter* spp. from leafy herbs (25.1%) and green onions (20.4%), *K. pneumoniae* from cucumbers (13.5%), green onions (13.3%), berries (13.2%) and tomatoes (13%), *P. aeruginosa* from green onions (11.7%), berries (7.5%) and leafy vegetables (6.5%), *Acinetibacter* spp. from tomatoes (3.4%), cucumbers (3.4%) and green onions (3.2%).

According to the literature, vegetables produced in or close to soil - such as leaf vegetables are at special risk for contamination with soil-borne bacteria, either belonging to natural soil microbiota or introduced into soil by manure fertilization [18]. In 2007, the FAO and WHO convened an expert committee to establish priority commodities of concern in terms of microbiological hazards associated with fresh produce and leafy vegetables and leafy herbs were given the highest level of priority, followed by berries, green onions, melons, sprouted seeds and tomatoes [4].

In our study the overall proportion of extended spectrum beta-lactamase (ESBL) production among Enterobacteriaceae was 36.8% and of methicillin-resistance in *S. aureus* (MRSA) 10.7%. The prevalence of ESBL production among *E. coli* isolates was significantly higher than in *K.pneumoniae.* Vancomycin resistance was observed in 3.1% of isolated enterococci (VRE). Carbapenem resistance was identified in 35.3% of *P.aeruginosa* isolates and 66.8% of *Acinetibacter* spp. isolates. Resistance to third-generation cephalosporins was observed in 9.7% *K.pneumoniae* and *E.coli* in 14.2% isolates.

The potential for transfer of antimicrobial-resistant bacteria from fresh vegetables into the human population is cause for concern. However, to date, few studies have focused on the occurrence of antimicrobial-resistant commensally bacteria in fresh vegetables sold at retail. Several studies are accessible regarding major antimicrobial-resistant bacteria (e.g., Enterobacteriaceae, *Pseudomonas* spp., Gram-positive cocci). These studies revealed vegetables to be a potential-although rare-vector for extended-spectrum beta-lactamase-producing Enterobacteriaceae, colistin- and carbapenem-resistant *Pseudomonas aeruginosa*, linezolid-resistant enterococci and staphylococci, and vancomycin-resistant enterococci [18]. Resistance data for isolates differed significantly between studies; this might be related to the very different geographical locations, the different nature of fresh vegetable samples, and the different choice of antibiotics and breakpoints. Korean leafy vegetables were contaminated with MRSA strains [19]. Vancomycin–teicoplanin-resistant enterococci were isolated from fresh produce in Korea [20]. Linezolid-resistant strains of *E. faecalis* and *E. faecium* were isolated from German vegetables [21]. Resistance in *E. faecalis* isolates from Portuguese ready-to-eat salad was restrained to tetracyclines and erythromycin [22]. One linezolid-resistant *E. faecium* was detected in Canadian vegetables as well [23]. From Tunisia [24] reported resistance *Enterococcus* species to high level concentrations of aminoglycosides and chloramphenicol. Gómez-Aldapa *et al.* [25] reported 100% resistance to amikacin and colistin in *E. coli* pathotypes from Mexican cactus salads. Resistance in *E. coli* isolates from raw vegetables at a retail market in the Czech Republic was restrained to tetracyclines [26]. One study identified significantly higher resistance rates to aminoglycosides in *Pseudomonas* isolates from fruit vegetables compared with root vegetables or salads [21].

The results this study adds to the global knowledge base regarding the prevalence and characteristics of antibiotic-resistant bacteria in fresh vegetables required for future risk analysis. Prevalence of antimicrobial resistance among gram-positive and gram-negative organisms, the ability of antibiotic-resistant bacteria to persist in hospitals and the environment, and possible transfer of resistance genes from this pathogens to other endogenous human flora or pathogens is reason for concern in the health care setting. Therefore, the impact of contamination of fresh vegetables with antibiotic-resistant pathogens and prophylactics and their possible cross-resistance with antimicrobials used to treat human infections should be investigated further with the goal of reducing the number of infections and fatalities associated with bacterial infections.

CONCLUSIONS

The results of this study indicate that the contamination of fresh vegetables with antibiotic-resistant bacteria at levels representing a risk to public health was found to be frequent and widespread occurrence in the Ukrainian marketplace. This trend extended to both imported and domestic fresh vegetables, and was particularly strong in domestic organic produce. This finding suggests that food safety practices carried out by the different players along the food supply chain, from agricultural practices by the farmers to handling practices by the food distributors

and vendors are generally not sufficient. Food producers, distributors and vendors should minimize the risk of contamination of fresh vegetables with antibiotic-resistant bacteria, and ensure in the production, transportation, storage and sale of fresh vegetables of acceptable quality and safety. It is necessary to ensure provide food safety oversight of the regulated parties and to promote safe production and handling of foods throughout the entire food production chain.

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ORIGINAL ARTICLE

THE BALANCE OF IL-1B, IL-10 AND THE LEVEL OF IKBA EXPRESSION IN CHILDREN WITH CHRONIC CATARRHAL GINGIVITIS AND GASTRODUODENITIS

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ABSTRACT

The aim: To define the level of pro- and anti-inflammatory IL-1β, IL-10 in the oral fluid of children with chronic gastroduodenitis, depending on the level of IkBα expression.

Materials and methods: Studiing the oral fluid and scraping of the gums of 50 children 6-12 years old was carried out to determine the level of IL-1β, IL-10 and IkBα. The children were divided into 3 groups. Group 1 - 10 children with healthy periodontium and without somatic diseases. 2nd group - 20 somatically healthy children with chronic catarrhal gingivitis. The third group (20 children) of children with gastroduodenitis and chronic catarrhal gingivitis. The determination of the mRNA expression of the IkBα gene and the level of IL-10 and IL-1β in the oral fluid was carried out by real-time PCR.

Results: We determined that the level of pro-inflammatory IL-1β in the oral fluid of primary school children had different levels in accordance with the state of dental and somatic health. It was the lowest in somatically healthy children without signs of gingivitis. We revealed that the level of IL-10 has anti-inflammatory properties, and an inverse relationship with the concentration of IL-1β in the oral fluid of examined children. Analysis of IkBα expression made it possible to reveal its suppression in children of the second and third groups compared with healthy children.

Conclusions: We found changes in the levels of IkBα in the gums of children with chronic catarrhal gingivitis; attenuated IkBα expression may lead to the deregulation of NF-κB pathways in the pathogenesis of periodontitis. Decreased IkBα expression may affect cytokine production and inflammatory response associated with chronic catarrhal gingivitis and with chronic gastroduodenitis.

KEY WORDS: children, gingivitis, gastroduodenitis, cytokines, IkBα

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INTRODUCTION

Lesions of the gastrointestinal tract (chronic gastroduodenitis) occupy one of the leading places among other general somatic pathologies. Chronic gastroduodenitis plays great role in the development of gingivitis and periodontitis [1,2,3,4].

The pathogenetic commonality of many general somatic processes and inflammatory diseases of the oral cavity can be explained by similar mechanisms of cellular damage for the whole organism. The cytokine profile of immunobiological processes plays the leading role in the occurrence of these changes [5].

The level of cytokines reflects the pro- and anti-inflammatory activity of any inflammatory process [6]. Cytokines are subdivided into pro-inflammatory (they are involved in the initiation of inflammation), and anti-inflammatory, depending on the nature of the effect on the inflammatory process. The key pro-inflammatory cytokine is interleukin-1 (IL-1), the main anti-inflammatory cytokine is interleukin-10 (IL-10) [7].

Recent studies show that the level of cytokines in saliva does not correlate with their level in the blood. This fact indicates a certain independence of the local immunity of the oral cavity, and reflects the general tendencies of the cytokine cascade in the patient's body [8].

Nuclear factor-kB (NF-kB) is a cytokininducible factor that plays a significant role in the transcriptional regulation of genes involved in inflammatory reactions and cell survival [9,10].

NF-κB is associated with many autoimmune diseases, chronic inflammation, metabolic disorders [11]. Normally, NF-kB is present in the cytoplasm in an inactive form due to its complex with IkB, which prevents the penetration of NF-kB into the nucleus. Regulation of the NF-kB and the IkB interaction plays a key role in NF-kB. NF-kB activation is initiated by extracellular signals, recorded by membrane receptors, and transmitted into the cell. In addition, IκBα blocks the ability of NF-κB transcription factors to bind to DNA, which is necessary for the correct functioning of NF-κB [12]. Understanding the molecular mechanisms that regulate NF-κB signaling and its functioning is important for finding new approaches in the treatment of gingivitis and periodontitis.

THE AIM

To study and to compare the level of pro- and anti-inflammatory IL-1β, IL-10 in the oral fluid of children with chronic gastroduodenitis, depending on the level of IkBα expression.

MATERIALS AND METHODS

The oral fluid and scraping of the gums of 50 children 6-12 years old were studied to determine the level of IL-1β, IL-10 and IkBα. Children were divided into 3 groups. Group 1 (control) consisted of 10 schoolchildren, who had a healthy periodontium according to a clinical dental examination and did not have somatic diseases according to a pediatrician examination. Group 2 consisted of 20 children and they had chronic catarrhal gingivitis, they were somatically healthy. The third group (20 children) had chronic gastroduodenitis and chronic catarrhal gingivitis. Children with chronic gastroduodenitis had an inpatient treatment at the Poltava Regional Children's Clinical Hospital and were treated according to the order of the Ministry of Health of Ukraine No. 59 dated January 29-th, 2013 «An approval of unified clinical protocols for medical care for children with digestive diseases.»

Dental examination was carried out according to the WHO method, 1989, the results were recorded in the examination cards. The the severity of gingivitis was assessed by the papillary-marginal-alveolar index (PMA) modified by Parma, 1960. The papillary bleeding index (PBI) was also determined as an indicator of the severity of gum inflammation. This index is assessed in 30 seconds after probing the interdental area. 1 degree - single punctate bleeding; 2 degree - linear / punctate mild bleeding along the edge of the apex of the papilla; 3 degree - moderate bleeding from the interdental papilla (in the form of triangle); 4 degree-severe bleeding that occurs immediately after probing the gum in the interdental spaces.

The level of IkBα expression was determined in the material by scraping the marginal part of the gum. The epithelium was taken from the marginal part of the gums with a disposable plastic spatula early in the morning (before the meal). The tip of a disposable spatula was cut off with sterile scissors and put to sterile eppendorf. The samples were stored and transported within 2 hours. These eppendorfs were transported to the laboratory in thermal containers with refrigerant.

The level of mRNA expression of the IkBα gene was determined by real-time PCR. Total RNA was isolated from a biological sample using the RIBO-sol-V reagent kit (AmpliSens, Russia). A set of reagents for carrying out the reverse transcription reaction (Syntol, Russia) was used to obtain cDNA.

The determination of the mRNA expression of the IkBα gene was carried out by real-time PCR using a DT-Light detector (DNA-Technology, Russia).

The level of IL-10 in the oral fluid was determined to characterize inflammation in the organs of the oral cavity. Unstimulated oral fluid was taken at fixed time, in the morning, on an empty stomach. Patients were previously offered to rinse the mouth. The collection of oral fluid was made by spitting 4 ml into plastic sterile tubes. They were hermetically closed and carried out in 30 minutes. This oral fluid was delivered to the laboratory.

The determination of IL-10 and IL-1β in the oral fluid was carried out by polymerase chain reaction using the «Interleukin-10-IFA-BEST», «Interleukin-1-IFA-BEST» kits.

The measurement of optical density (it is automatically converted to concentration) was carried out at a wavelength of 450 mm on a STATFax 303 Plus enzyme immunoassay analyzer (USA). The research results were processed using the generally accepted methods of medical statistics.

Table I. Condition of periodontal tissues in children

Contingent of children	Number of children	Papilla_bleeding index (PBI)	PMA
Healthy children	10	1,0	0 %
Healthy children with chronic catarrhal gingivitis	20	1,35±0,11*	22,25±0,57*
Children with chronic gastroduodenitis and chronic catarrhal gingivitis	20	2,5±0,12** ***	34,85±1,23** ***

Notes:
* - the difference is significant when we compare group 1 and group 2, p <0.05
** - the difference is significant when we compare group 1 and group 3, p <0.05
*** - the difference is significant we compare group 2 and group 3, p <0.05

Table II. The level of IL-1, IL-10 and expression of IKBa in elementary scool children

Contingent of children	Number of children	IL-1β, pg/ml	IL-10, pg/ml	IkBα $2^{-\Delta ct}$
Healthy children	10	3,72±1,12	1,44±0,1	0,045±0,07
Healthy children with chronic catarrhal gingivitis	20	183,82±15,76*	1,07±0,14*	0,022±0,003*
Children with chronic gastroduodenitis and chronic catarrhal gingivitis	20	282,33±6,82** ***	0,57±0,16** ***	0,026±0,04***

Notes:
* - the difference is significant when we compare group 1 and group 2, p <0.05
** - the difference is significant when we compare group 1 and group 3, p <0.05
*** - the difference is significant we compare group 2 and group 3, p <0.05

RESULTS AND DISCUSSIONS

The group of children with chronic gastroduodenitis had the most severe inflammation of gums (Table I). We determined that children with gastroduodenitis had chronic catarrhal gingivitis of moderate severity and the PMA index was 34.85 ± 1.23%. Somatically healthy children with chronic catarrhal gingivitis had mild severity of gum inflammation (22.25 ± 0.57%, p <0.05).

Children with chronic gastroduodenitis had significantly worse state of gum inflammation. It is also confirmed by high papillary bleeding index - 2.5 ± 0.12 points. Somatically healthy children had a low degree of PBI, namely, 1.35 ± 0.11 points.

Condition of periodontal tissues in children

The balance of pro- and anti-inflammatory IL-1β, IL-10, depending on the level of expression of IkBα is presented in Table II.

We determined that the level of pro-inflammatory IL-1β in the oral fluid of primary school children had different levels in accordance with the state of dental and somatic health. Thus, IL-1 β level was low in somatically healthy children without signs of gingivitis or periodontitis - 3.72 ± 1.12 pg/ml. We determined a significantly higher concentration of IL-1β in the group of children with chronic catarrhal gingivitis, which reached 183.82 ± 15.76 pg/ml, and it was almost 45 times higher. The level of IL-1β was 1.5 times higher and reached 282.33 ± 6.82 pg/ml in the third group of examined children with chronic gastroduodenitis and chronic catarrhal gingivitis.

We found an inverse relationship with the concentration of IL-1β and the level of IL-10 (it has anti-inflammatory properties) in the oral fluid of children of the examined groups. We determined the concentration of IL-10 was 1.44 ± 0.17 pg/ml in healthy children, and it fell to 1.07 ± 0.14 pg/ml in somatically healthy children with chronic catarrhal gingivitis (p <0.05), due to increase of the severity of gingivitis. The level of IL-10 dropped almost 2 times in children with chronic gastroduodenitis and chronic catarrhal gingivitis, compared with children of the 2nd group and 3 times compared with healthy children and amounted to 0.57 ± 0.16 pg/ml (p <0.05, p <0.05).

We found the suppression of IkBα expression in children of the second and third groups compared with healthy children (0.022 ± 0.003 2-Δct and 0.026 ± 0.04 2-Δct compared with 0.045 ± 0.07 2-Δct). So, both somatically healthy children and children with chronic gastroduodenitis and chronic catarrhal gingivitis had a lower level of IkBα expression (p <0.05).

CONCLUSIONS

Our results demonstrate changes in IkBα levels in the gums of children with chronic catarrhal gingivitis. We suggest that attenuated IkBα expression may contribute to deregulation of NF-κB pathways in the pathogenesis of gingivitis and periodontitis. Decreased IkBα expression may affecton cytokine production and inflammatory response associated with chronic catarrhal gingivitis in children with chronic gastroduodenitis.

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ORIGINAL ARTICLE

PARTICULAR QUALITIES OF THE PROTEOLYTIC SYSTEM IN PATIENTS WITH TUBERCULOSIS DEPENDING ON THE SENSITIVITY OF THE PATHOGEN

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ABSTRACT

The aim: Studying the features of the proteolytic system in patients with tuberculosis depending on the sensitivity of the pathogen.

Materials and methods: In the course of the research we studied the level of elastase in the blood of 111 patients. The first group consisted of 66 (59.5%) people with pulmonary tuberculosis (39 were sensitive to antibacterial drugs, 27 were resistant). The second group included 13 (11.7%) patients with tuberculous pleurisy. The third group consisted of 32 (28.8%) patients with dual localization of the process (pulmonary tuberculosis and pleural tuberculosis).

Results: The level of neutrophil elastase in patients with tuberculous pleurisy (253.2 nmol / min • ml) was 2.2 times higher than in patients with sensitive pulmonary tuberculosis (110.1 nmol / min • ml) and higher than in patients with resistant pulmonary tuberculosis 3.0 times. In combined pulmonary and pleural tuberculosis (third group) the level of elastase was 1.6 times higher than in pulmonary tuberculosis (176.9 nmol / min • ml)) ($p < 0.01$), but lower than in pleurisy in 1, 4 times. In sensitive combined tuberculosis (lungs and pleura) the level of NE was 1.5 times higher than in patients of subgroup 1a ($p < 0.01$) and 1.4 times lower than in patients with tuberculous pleurisy ($p < 0.01$).

Conclusions: The highest level of elastase in tuberculous pleurisy can be explained by its increased production, contributes to increased "permeability" of the pleural sheets and the accumulation of pleural effusion. In resistant forms of tuberculosis, the immune response in the form of the activity of the proteolytic system, which is lower than in sensitive forms, can be explained by the exhaustion of the immune system under the influence of aggressive tuberculosis. The above can be associated with both the weakening of the patient's body and the aggressiveness of the pathogen.

KEY WORDS: elastase, pulmonary tuberculosis, pleural tuberculosis

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INTRODUCTION

Despite the fact that tuberculosis is a curable disease, it is one of the ten causes of death in the world [1]. There was a decrease in the incidence of tuberculosis in Ukraine, in 2019 (from 62.3 to 60.1 per 100 thousand of the population) and mortality (from 9.4 to 8.8 per 100 thousand of the population) from this disease. At the same time, the incidence of children increased by 1.1%, and the incidence of adolescents – by 5.3% [2]. At the same time, the rate of increasing the effectiveness of treatment of TB patients in Ukraine is insufficient to achieve the WHO target (by 2025 there should be 90% of patients who have successful treatment). Among patients with sensitive tuberculosis, about 77% had effective treatment. Only 49% of patients with multidrug-resistant tuberculosis (MDR-TB) had a satisfactory treatment outcome. Only 37% of patients with extended resistance (XDR-TB) were successfully cured [1].

The authors had a research involving 796 patients with tuberculosis. It was found that the main barriers to tuberculosis treatment in Ukraine encompass structural barriers associated with the organization of the health care system and the unregulated regulatory framework, barriers at the level of anti-tuberculosis institutions and the general route of the patient between different branches of health care, gaps in interaction between health workers and patients, individual factors, including social, economic and psychological consequences of treatment for the patient [1].

However, there is insufficient data on the pathogenesis of tuberculosis, depending on the sensitivity of the pathogen and the characteristics of the proteolytic system.

THE AIM

Studying the features of the proteolytic system in patients with tuberculosis depending on the sensitivity of the pathogen.

MATERIALS AND METHODS

In the study, we examined 111 patients who were divided into three groups. The first group consisted of 66 (59.5%) people with pulmonary tuberculosis (PTB) – (Table I). The second group included 13 (11.7%) patients with tuberculous pleurisy (PLTB). The third group consisted of 32 (28.8%) patients with dual localization of the process (pulmonary tuberculosis and pleural tuberculosis) – (PTB-PLTB). The main last group consisted of 23 healthy individ-

Table I. Distribution of examined patients according to the sensitivity of the pathogen

Group patients	Sensitive		Resistant		Total	
	quantity	%	quantity	%	quantity	%
The first	39	59,1	27	40,9	66	59,5
The second	13	100,0	-	100,0	13	11,7
The third	22	68,8	10	31,2	32	28,8
Total	**74**	**66,7**	**37**	**33,3**	**111**	**100,0**

Table II. The level of neutrophil elastase in patients of the first group

Indicator	Groups of patients				
	1a group (n=39)		16 group (n=27)		Control group [&] (relatively healthy individuals) (n=23)
Neutrophil elastase, nmol / min • ml	Fluctuations of values	Average value	Fluctuations of values	Average value	75,0
	35,2-215,1	110,1±11,8[*,#]	34,6-163,1	78,4±9,2*	

Note:
* – statistically confirmed differences between the indicators of patients of groups I and II ($p<0,05$);
# – statistically confirmed differences between the indicators of the studied patients and the control group ($p<0,01$);
& – according to previous studies [4].

Table III. The level of neutrophil elastase in patients of the second group

Indicator	Patients with pleurisy (n=13)		Control group (n=23)
	Range	Average value	
Neutrophil elastase, nmol / min • ml	144,7 – 350,5	253,2±16,7*	75,0

* – statistically confirmed differences between the indicators of the study of patients and the control group ($p<0,01$).

uals who had no lung or pleural disease at the time of the examination and had not been ill in the past. Among the examined control group there were 11 women and 12 men.

When studying the sensitivity of the mycrobacterium tuberculosis (MBT) to antibacterial drugs, it was found that out of 39 (59.1%) people on PTB had preserved sensitivity of mycobacteria to first-line drugs. These patients were assigned to subgroup 1a. The other 27 (40.9%) patients had MDR-TB, that is, resistant to the two main first-line anti-TB drugs: isoniazid and rifampicin. These patients were assigned to subgroup 1b. Among patients of subgroup 1b, in addition to the MBT resistance to rifampicin and isoniazid, resistance to ethambutol was found in 17 (63.0%) patients, to pyrazinamide in 13 (48.1%) patients, and in all 100.0% of patients to streptomycin. In addition, resistance to kanamycin, capreomycin and amikacin was found in 4 (14.8%) patients, 7 (25.9%) and 17 (63.0%), respectively. Resistance to levofloxacin was stated in 7 (25.9%) patients, to ofloxacin – in 6 (22.2%) and to moxifloxacin in 7 (25.9%) subjects. Thus, in 7 patients, in addition to MBT resistance to rifampicin and isoniazid, a combination with one of the injectable drugs and one of the fluoroquinolones was established. So, the expanded resistance of MBT to antibacterial drugs in patients of the first group occurred in 25.9% of those studied.

In the second group, all 13 (100.0%) patients were diagnosed with pleural TB with preserved sensitivity to the first-line drugs (by the genetic molecular method using the GeneXpert apparatus).

Among patients of the third group (PTB-PLTB) patients with pulmonary and pleural tuberculosis sensitive to anti-tuberculosis drugs predominated – 22 (68.8%), which were designated as subgroup 3a. There were 10 patients (31.2%) with resistant tuberculosis, which were assigned to subgroup 3b. In this subgroup (3b) in addition to resistance to isoniazid and rifampicin in 7 (70.0%) there was resistance to ethambutol, in 9 (90.0%) – to streptomycin, in 2 (20.0%) – to pyrazinamide, 1 (10.0%) – to capreomycin, kanamycin, ofloxacin moxifloxacin and ethionamide. Extended resistance occurred in 2 (20.0%) people.

In general, among the studied patients of all groups, tuberculosis sensitive to antibacterial drugs was stated in 74 (66.7%) patients, resistant – in 37 (33.3%) people.

The study of the proteolytic system was carried out by determining the activity of neutrophil elastase in the blood serum of patients by the spectrophotometric method, preliminarily freeing it from the complex with a proteinase inhibitor using Tris-HCl buffer. N-BOC-L-Alanine p-nitrophenyl ester was used as a substrate according to a known technique [3].

Table IV. The level of neutrophil elastase in patients of the third group

Indicator	Group of patients				
	3a subgroup (n=22)		3b subgroup (n=10)		Control group (n = 23)
	Fluctuations of values	Average value	Fluctuations of values	Average value	
Neutrophil elastase, nmol / min • ml	62,5-309,8	176,9±17,4*	62,5-291,4	173,8±26,9*	75,0

* – statistically confirmed differences between the indicators of the studied patients and the control group (p <0,01).

Table V. Comparison of the level of NE depending on the sensitivity of the pathogen

Study groups	The average value of NE, nmol / min • ml	
	Sensitive to antibacterial drugs tuberculosis, subgroup «a»	Resistant to antibacterial drugs tuberculosis, subgroup «b»
Group I	110,1±11,8	85,3±10,7
Group II	253,2±16,7	-
Group III	176,9±17,4	173,8±26,9
Total	155,7±10,4*	109,3±12,4*

* – statistically confirmed differences between the indicators of the studied patients (p <0,01).

This research was approved by the Ethics Committee of Sumy State University, Sumy, Ukraine (Protocol № 2, 03.11.2020). Research was conducted keeping to the main issues of the Convention of the Council of Europe on Human Rights and Biomedicine of Declaration of Helsinki of the World Medical Association on the ethical principles of conducting medical research involving human beings (1975, with further amendments, including version of 2000) and Order of Ukrainian Ministry of Health № 690 on 23.09.2009.

Statistical processing of the material was carried out using licensed software products included in the Microsoft Office Home & Business 2016 package (license X20-34344-01) on a personal computer Everest in Excel program by the method of variation statistics using the Student's test. The difference in indicators was considered reliable at p <0.05.

RESULTS AND DISCUSSION

We compared the level of neutrophil elastase (NE) in patients of both subgroups with the level of NE in relatively healthy individuals who at the time of the study had no diseases and had no lung diseases in the past (Table II). It was found that in patients with sensitive forms of pulmonary tuberculosis, the level of elastase ranged from 35.2 to 215.1 nmol / min • ml, on average it was 110.1 ± 11.8 nmol / min • ml. The level of NE in patients of subgroup 1a (110.1 ± 11.8) exceeded its value in healthy individuals by 1.5 times, which is significantly higher than its values in healthy individuals (p <0.01). In patients with multidrug-resistant tuberculosis (subgroup 1b), the level of elastase ranged from 34.6 to 163.1 nmol / min • ml with an average value of 78.4 ± 9.2 (p <0.05). The level of NE in patients of subgroup 1b exceeded its value in healthy individuals by 1.1 times, but this difference was insignificant (p> 0.05).

At the same time, the level of elastase in patients sensitive to antibacterial drugs pulmonary tuberculosis exceeded its level in patients with multi-resistant forms of tuberculosis by 31.7 nmol / min • ml, or 1.4 times (p <0.05).

In the second group, all 13 studied patients sensitive to antibacterial drugs pleurisy had a level of neutrophil elastase, which ranged from 144.7 – 350.5 nmol / min • ml (Table III). The mean value of elastasis was 253.2 ± 16.7 nmol / min • ml. In the case of the control group, the level of elastase were 3.4 times larger. Compared with the first (PTB) group, the level of elastase was 2.2 times higher than in subgroup 1a (pulmonary tuberculosis is sensitive to antibiotic therapy). That is, the level of neutrophilic elastase in patients with tuberculous pleurisy was significantly higher than in patients with pulmonary tuberculosis (p <0.01) and more than in individuals from the control group (p <0.05).

Among the patients of the third group, who were diagnosed with sensitive tuberculosis (subgroup 3a), the level of NE fluctuated within 62.5 – 309.8 nmol / min • ml, on average it was 176.9 ± 17.4 nmol / min • ml, then there is 2.4 times more than in the control group (p <0.01). Among the subjects of this group, in which there was resistant tuberculosis to antibacterial drugs (subgroup 3b), the level of NE was in the range of 62.5 - 291.4 nmol / min • ml, ie the average value of the indicator was not at the level of 173.8 ± 26.9 nmol / min • ml, which is 2.3 times more than in the control group (p <0,01). The average level of NE in subgroup 3a was 176.9 nmol / min • ml practically did not differ from the level of NE in subgroup 3b – 173.8 nmol / min • ml (p> 0.05) - (Table IV).

We compared the level of NE in patients of different groups with susceptible tuberculosis (Table V). In persons of subgroup 3a the level of NE was 1.6 times higher than in patients of subgroup 1a – 110.1 nmol / min • ml ($p < 0.01$) and 2.3 times less than in patients of group II – 253.2 nmol / min • ml ($p < 0.01$).

We also compared the level of elastase in multidrug-resistant tuberculosis in patients with PTB and PTB – PLTB (Table V). The level of NE in subgroup 3b differed significantly from subgroup 1b. Thus, in subgroup 1b of the subjects the level of NE was 85.3 nmol / min • ml, and in subgroup 3b was 173.8 nmol / min • ml, ie it was 2 times higher ($p < 0.01$).

The results show that in patients with pulmonary and pleural tuberculosis, sensitive to antibacterial therapy, the level of elastase significantly exceeded in those who did not have at the time of examination and in the past lung and pleural diseases. In pulmonary tuberculosis it was 1.6 times higher, and in patients with tuberculous pleurisy 3.4 times higher than in the control group. At the same time, when combining pulmonary tuberculosis with pleural, the level of elastase was 2.4 times higher than in the control group. Thus, the most active proteolytic system was in isolated pleural tuberculosis, and the pulmonary process significantly reduced this activity. In pulmonary tuberculosis, the activity of the proteolytic system was even lower.

Resistance of Mycobacterium tuberculosis in specific patients (multidrug-resistant tuberculosis) affected proteolytic activity by inhibition in patients of all groups (Table V).

CONLUSIONS

1) The level of neutrophil elastase in patients with tuberculous pleurisy is 2.2 times higher than in patients with pulmonary tuberculosis (110.1 nmol / min • ml) and higher than in combined pulmonary and pleural tuberculosis (176.9 nmol / min • ml) 1.4 times ($p < 0.01$). This can be explained on the one hand by the greater "use" of elastase in pulmonary forms of tuberculosis, and on the other – increased production of it in pleural tuberculosis, which contributes to increased "permeability" of the pleural leaves.
2) The level of neutrophil elastase in the combined process (lungs and pleura) is 2.4 times higher than in the control group, but almost no different from sensitive forms of pulmonary tuberculosis.
3) With sensitive joint tuberculosis (lungs and pleura), the level of NE was 1.5 times higher than in patients of subgroup 1a ($p < 0.01$) and 1.4 times less than in patients with tuberculous pleurisy ($p < 0.01$).
4) In multidrug-resistant pulmonary tuberculosis, the level of NE (85.3 nmol / min • ml) was 2 times lower than in combined tuberculosis (lungs and pleura) ($p < 0.01$). In resistant forms of tuberculosis, the immune response, in the form of activity of the proteolytic system, is lower than in sensitive forms, which can be associated with both the weakness of the body as a whole and the aggressiveness of the pathogen.

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ORIGINAL ARTICLE

PECULIARITIES OF CHANGES IN HOMOCYSTEINE LEVELS DEPENDING ON VITAMIN STATUS IN PATIENTS WITH CHRONIC PANCREATITIS AND TYPE 2 DIABETES

10.36740/WLek202101119

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ABSTRACT

The aim: To determine the peculiarities of changes in the homocysteine levels in the patients with chronic pancreatitis and type 2 diabetes blood serum depending on the vitamin status.

Materials and methods: We investigated 36 patients with chronic pancreatitis and type 2 diabetes, who were included in the first group of the patients examined; Group 2 consisted of 34 patients with chronic pancreatitis; and Group 3 of the patients examined consisted of 40 patients with type 2 diabetes.

Results: All patients examined were diagnosed with type 2 diabetes mellitus of moderate severity. Also, the diagnosis of chronic pancreatitis was confirmed in all patients with type 2 diabetes, which was manifested by exocrine pancreatic insufficiency according to the results of clinical, laboratory and instrumental methods of examination. There was a significant decrease in the level of all B vitamins and 25-(OH)D in patients with chronic pancreatitis and type 2 diabetes (Group I). An increase in the concentration of homocysteine in the serum in all examined groups of patients was established, with the maximum deviation from the norm in patients with chronic pancreatitis and type 2 diabetes (up to 32.7 ± 0.8 μmol / L <0.01). The correlation analysis revealed a strong direct relationship between the level of homocysteine and vitamins B12, B6, 25-(OH)D and an inverse correlation between vitamin B9 in the group of patients with chronic pancreatitis and type 2 diabetes.

Conclusions: Patients with chronic pancreatitis and type 2 diabetes have a decreased levels of B vitamins (B1, B6, B9, B12) and 25-(OH)D, which is accompanied by an increase in serum homocysteine. In patients with chronic pancreatitis and type 2 diabetes, the level of homocysteine in the blood serum directly depends on the decrease in the levels of vitamins B6, B12 and 25-(OH)D in blood serum, as well as inverse depends on vitamin B9 levels in these patients.

KEY WORDS: chronic pancreatitis, type 2 diabetes mellitus, vitamins, homocysteine

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INTRODUCTION

Currently both diabetes mellitus (DM) and chronic pancreatitis (CP) pose a serious health and social problem due to the increasing prevalence and tendency towards progressive growth. The prognosis of diabetes and life expectancy are determined by the rate of development and progression of complications that occur as a result of diabetes. In this case, the higher the comorbidity in diabetes, the higher the risk of developing complications that aggravate each other [1].

Chronic pancreatitis is one of the most complex polyetiological and polymorbid diseases. The prevalence of CP among the population of different countries varies from 0.2% to 0.7% and reaches 6.0-9.0% among patients with gastrointestinal diseases [2]. Summation and potentiation of adverse effects of CP risk factors and causal factors contribute to the growth of CP incidence and its progressive course, with exocrine pancreatic insufficiency, development of maldigestion, malabsorption and trophological insufficiency in patients with impaired metabolism of all kinds [3, 4].

Studies of the pathogenesis of CP with multiple comorbidities prove the important role of such factors as systemic inflammation, imbalance of pro- and anti-inflammatory cytokines, factors of proteinase-inhibitory system with increasing negative effects of systemic proteolysis, violation of oxidant-antioxidant homeostasis with the activation of oxidative and nitrosive stress associated with natural antioxidants deficiency, hyperglycemia associated with the formation of pancreatogenic diabetes mellitus, which increases due to concomitant intestinal dysbiosis. The CP clinic with concomitant intestinal dysbiosis is complicated by increased intestinal dyspepsia, indigestion (steatorrhea, malabsorption of fat-soluble vitamins D and K and water-electrolyte imbalance) and astheno-vegetative syndrome caused by hypo- and avitaminosis, dysproteinemia and intoxication [5, 6].

Currently, the increase in homocysteine levels is given special attention due to the possibility of atherothrombotic, cerebral, cardiac and peripheral vascular disorders, as well as neurodegenerative processes [1]. Especially relevant is the study of homocysteine effect on the formation of complications, including vascular micro- and macroangiopathies in metabolically associated diseases, such as type 2 diabetes, especially in combination with CP.

Homocysteine is a sulfur-containing amino acid that is formed during the metabolism of methionine and cysteine.

In the body, methionine is metabolized by demethylation, resulting in S-adenosylhomocysteine converted to homocysteine. The donor of the methyl group in this case is trimethylglycine (betaine). Another way of remethylation of homocysteine under the action of methionine synthetase is also possible. This reaction requires vitamin B12, which is a coenzyme of methionine synthetase. Homocysteine can also be catabolized in the process of trans-sulfurization by conversion to cysteine through cystathionine with the participation of the enzyme cystathionine-beta-synthetase, and the coenzyme required in this reaction is vitamin B6 [1].

Therefore, the study of vitamin status of the body and its possible impact on the level of homocysteine in patients with CP, especially in combination with type 2 diabetes is a topical issue in comorbid patient treatment.

THE AIM

To determine the peculiarities of changes in the homocysteine levels in the patients with CP and type 2 diabetes blood serum depending on the vitamin status.

MATERIALS AND METHODS

We investigated 36 patients with CP and type 2 diabetes, who were included in the first group of the patients examined (20 men (55.6%) and 16 women (44.4%) at an average age of 48.3 ± 5,1 years). Group 2 consisted of 34 patients with CP (21 men (61.8%) and 13 women (38.2%) at an average age of 46.7 ± 4.2 years); and Group 3 of the patients examined consisted of 40 patients with type 2 diabetes (22 men (55.0%) and 18 women (45.0%) at an average age of 48.9 ± 6.3 years). All examined patients were either hospitalized in the Endocrinology and Gastroenterology Departments of Municipal Non Profit Enterprise "Transcarpathian Regional Clinical Hospital Named After Andrii Novak" of Transcarpathian Regional Council or were on outpatient observation by the district family doctors at the place of their residence.

The control group included 20 healthy individuals (11 men (55.0%) and 9 women (45.0%) at an average age of 45.2 ± 5.1 years).

All studies were performed with the consent of the patients, and their methodology was in line with the Helsinki Declaration of Human Rights of 1975 and its revision of 1983, the Council of Europe Convention on Human Rights and Biomedicine and the current legislation of Ukraine.

All patients were examined sing general clinical, anthropometric, instrumental and laboratory research methods. All patients also underwent ultrasound examination of the abdominal cavity by conventional methods. Standard general and biochemical studies of blood serum were conducted with an emphasis on carbohydrate metabolism (glucose, glycosylated hemoglobin, insulin, C-peptide, proinsulin and glucose tolerance test).

All examined patients had their blood serum tested for B vitamins, namely: vitamin B1 (thiamine) and vitamin B6 (pyridoxine) were tested by high performance liquid chromatography (HPLC) using Agilent 1100 Fluorescence Detector (Recipe Complete Kit, Germany); vitamin B9 (folic acid) was tested by immunochemical method with electrochemiluminescence detection (ECLIA Cobas 6000), using the test systems of Roche Diagnostics (Switzerland), and vitamin B12 (cyanocobalamin – holotranscobalamin) was tested using immunochemical chemiluminescent detection (CLIA Architect) with the test systems of Abbot Diagnostics (USA).

Studies of 25-hydroxyvitamin D, 25-(OH)D levels were performed to assess the status of vitamin D in the examined patients using an immunochemical method with electrochemiceluminescent detection (ECLIA Cobas 6000), using Roche Diagnostics test systems. The level <20 ng / mL was evaluated as vitamin D deficiency; 20.0 <30 ng / mL was considered insufficient, and the level ≥ 30 ng / mL was evaluated as optimal.

Serum homocysteine levels were also determined with Cobas 8000 test system (Roche Diagnostics).

The diagnosis of CP was established in accordance with the Marseille-Roman criteria (1989) with the addition of Ya.S. Zimmerman (1995) and clarifications of ICD-10. A coprological study was performed, serum amylase and fecal elastase-1 levels were determined, and ^{13}C-mixed triglyceride (^{13}C-CTDT) and ^{13}C-amylase respiratory tests (^{13}C-ADT) were used to study the exocrine function of the pancreas,.

Medical care was provided to the examined patients with diabetes according to the clinical protocols of the Ministry of Health of Ukraine and local protocols. The diagnosis of type 2 diabetes was established in accordance with the recommendations of the IDF (2005), as well as taking into account the criteria of a unified clinical protocol (The Ministry of Health of Ukraine Order of 21.12.2012 № 1118) [7, 8]. The severity of type 2 diabetes was assessed by the level of HbA1c (Normal: up to 6.0%).

The analysis and processing of the results of the examination was carried out by means of the STATISTICA 10.0 computer program (StatSoft Inc., USA) using parametric and nonparametric methods for evaluating the results.

RESULTS AND DISCUSSION

All patients examined were diagnosed with type 2 diabetes mellitus of moderate severity. Also, the diagnosis of chronic pancreatitis was confirmed in all patients with type 2 diabetes, which was manifested by exocrine pancreatic insufficiency according to the results of clinical, laboratory and instrumental methods of examination (data of coprological study, changes in serum amylase levels, fecal elastase ZTDT and ^{13}C-ADT).

Patients with CP and type 2 diabetes were tested for vitamin B and 25-(OH)D and serum homocysteine levels. The results are presented in table 1.

There was a significant decrease in the level of all B vitamins and 25-(OH)D in patients with CP and type 2 diabetes (Group I). In particular, the most pronounced decrease was found in the levels of vitamins B9 and B12. Thus, they were reduced 4.7 and 3.5 times compared with

Table 1. Changes in vitamin and homocysteine levels in the patients examined

Indicator (reference values)	Control group	Patients examined		
		Group I (n=36)	Group II (n=34)	Group III (n=40)
Vitamin B1, mcg/L (over 49)	73.2±2.4	32.1±2.8 **	51.7±4.4 *,+	41.3±3.2 *
Vitamin B6, mcg/L (8.7-27.2)	18.5±1.7	6.3±0.7 **	8.1±0.3 *	7.7±0.5 *
Vitamin B9, ng/mL (4.6-18.7)	14.5±0.9	3.1±0.4 **	4.7±0.5 **	4.0±0.7 **
Vitamin B12, pg/mL (197.0-771.0)	524.1±9.5	148.4±10.1 **	223.3±9.9 *,+	186.8±9.4 *
25-(OH)D, ng/mL, (over 30)	47.2±2.7	19.3±0.7*	23.1±1.4 *	29.8±1.4 +
Homocysteine, μmol / L (less than 15.0)	9.7±0.6	32.7±0.8 **	17.1±0.5 *,+	24.3±0.5 *

Note: the differences between the indicators in the control group and the examined patients are significant: * $p < 0.05$; ** $p < 0.01$; differences between the indicators in the examined patients of Groups I and II are significant: + $p < 0.05$.

Table 2. Comparison of B vitamins with homocysteine levels in the examined patients

Vitamins level	Serum homocysteine levels		
	Group I	Group II	Group III
Vitamin B1	r= 0.46; p<0.05	_	-
Vitamin B6	r= 0.72; p<0.01	r= 0.58; p<0.05	r= 0.62; p<0.05
Vitamin B9	r= -0.84; p<0.01	_	r= 0.44; p<0.05
Vitamin B12	r= 0.90; p<0.01	_	r= 0.84; p<0.01
25-(OH)D	r= 0.74; p<0.01	_	r= 0.58; p<0.05

this indicator of the control group ($p < 0.01$). The levels of vitamins B6 and B1 were also lower compared to those in the control group – 2.9 and 2.3 times, respectively ($p < 0.01$).

In the comparison groups, more pronounced changes in the levels of B vitamins were found among patients with type 2 diabetes (Group III). At the same time, the same tendency was observed as in patients of Group I, i.e. the maximum decrease in the level of vitamin B9 (3.6 times – $p < 0.01$) and B 12 (2.8 times – $p < 0.05$).

Patients with CP (Group II) also manifested a decrease in the concentration of B vitamins. In addition, it should be noted that the level of vitamin B9, although lower than in the control group (4.7 ± 0.5 ng / mL and 14.5 ± 0.9ng / mL, respectively, $p < 0.01$), did not go beyond the reference.

The analysis of vitamin D status in the examined patients also indicates its maximum decrease among patients of Group I (patients with CP and type 2 diabetes). It should be noted that the level of 25-(OH)D in the comparison groups was reduced more significantly in patients with CP (Group II) than with type 2 diabetes (Group III).

An increase in the concentration of homocysteine in the serum in all examined groups of patients was established, with the maximum deviation from the norm in patients with CP and type 2 diabetes (up to 32.7 ± 0.8 μmol / L < 0.01).

The correlation analysis revealed a strong direct relationship between the level of homocysteine and vitamins B12, B6, 25-(OH)D and an inverse correlation between vitamin B9 in the group of patients with CP and type 2 diabetes (Table 2).

In patients with type 2 diabetes (Group III) a strong correlation was found only between homocysteine and vitamin B12, while the dependence of the average intensity was established between the levels of vitamins B6 and 25-(OH)D. For patients of Group II (patients with CP) the dependence was only fond between changes in homocysteine and vitamin B6 levels.

Therefore, in patients with CP, especially in combination with type 2 diabetes there is a decrease in the level of both water-soluble (B vitamins – B1, B6, B9, B12) and fat-soluble (25-(OH)D) vitamins due to violation of exocrine insufficiency of the pancreas, malabsorption, as well as dysmetabolic disorders. Decreased levels of folic acid, pyridoxine, cyanocobalamin, which is one of the main substrates for the formation of homocysteine, contribute to its increase in blood serum. It is assumed that the deficiency of B vitamins with the subsequent development of hyperhomocyteinemia can trigger a cascade of vascular complications in patients with CP and type 2 diabetes. It is known that an increase in homocysteine levels is an independent risk factor for the development of different complication, including osteoporosis, indicated by a decrease in the level of 25-(OH)D in patients with CP type 2 diabetes combination.

CONCLUSIONS

1. Patients with CP and type 2 diabetes have a decreased levels of B vitamins (B1, B6, B9, B12) and 25-(OH)D,

which is accompanied by an increase in serum homocysteine.
2. In patients with CP and type 2 diabetes, the level of homocysteine in the blood serum directly depends on the decrease in the levels of vitamins B6, B12 and 25-(OH) D in blood serum, as well as inverse depends on vitamin B9 levels in these patients.

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ORIGINAL ARTICLE

PEQULIARITIES OF MORPHOGENESYS AND TOPOGRAPHY OF INFRAHYOID TRIANGLES IN HUMAN PREFETUSES AND FETUSES

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ABSTRACT

The aim: To investigate morphology and developmental features of anatomical structures in the infrahyoid triangles of human neck during prefetal and fetal periods of human ontogenesis.

Materials and methods: We have studied 30 specimens of human prefetuses from 7th till 12th week (16,0-82,0 mm of parieto-coccygeal length (PCL)) and 30 human fetuses aged from 4th till 10th month (84,0-360,0 mm PCL) of intrauterine development by the means of macro-, microscopy, morphometry, three-dimensional remodeling and statistical analyses.

Results: We can observe anterior triangle in human fetuses after the time when common precursor muscular mass splits into two: the anterior and posterior portions which will give rise to the sternocleidomastoid and trapezoid muscles accordingly. The area index of the central triangle in human fetuses 4th – 10th month of intrauterine development shows the increasing tendency with the highest rates at 8th–10th months period – 1100-1200 mm^2. The angulated course of omohyoid muscle is visible at late prefetal and early fetal periods (3-4th month; 80,0-130,0 PCL) as well as the presence of intermediate tendon. Peaks of the area of sternocleidomastoid region area evaluation was observed in 190,0-210,0 mm PCL and 260,0-270,0 mm PCL human fetuses; 6th and 7,5th months accordingly.

Conclusions: The critical periods for the AT and SCM regions should be considered 6th and 8th months of the IUD. Prefetal period shows the presence of AT and border structure for the IH neck – precursor of HB. Fetal period of IUD (4th – 10th months of IUD) should be considered as such that represents an adult-alike morphology of IH neck: presence of IH triangles with fully developed vascular, muscular and fascial content.

KEY WORDS: human prenatal development, neck of human fetus, infrahyoid region, neck morphogenesis

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INTRODUCTION

The spaces that are found in the infrahyoid region are the clinical landmarks for possible spreading diseases of inflammatory (reactive adenopathy or the soft tissues inflammation), odontogenic, traumatic or oncological origins [1, 2, 3]. For instance, these may be oncological metastases (squamous carcinoma of larynx, lymphoma, thyroid cancer), Zenker's diverticulum, thyroglossal duct cyst etc. [4, 5]. Moreover, braches of nerve supply such as recurrent laryngeal nerve may be often damaged during thyroid surgeries [6]. One of the most common severe complications in the deep infrahyoid region is septic thrombosis of the internal jugular vein (IJV) [2]. Infrahyoid region of neck is also found to tend in high frequency of congenital malformations like thyroglossal duct cyst, lymphangiomas and branchial cleft cysts [3, 7]. Because all congenital malformations are formed from embryological tissue, knowledge on normal morhogenesis will allow to understand better possible ways of their formation and further effective treatment.

Infrahyoid muscles are used in surgery as flaps for reconstruction procedures of the larynx, esophagus and tongue [8]; laryngopharyngeal reconstruction in patients with pyriform sinus carcinoma [5]. The infrahyoid myocutaneous flap, fed by the superior thyroid artery and with double venous drainage, is used as a reliable and convenient technique for over 40 years [9, 10] by surgeons with different modified methods of incisions and flap preservation. We believe that detailed studies of the infrahyoid region in the focus of human prenatal development may help to improve surgical techniques of reconstructive surgeries in children and develop additional prenatal diagnostic indexes for possible congenital malformations detection.

THE AIM

To investigate and circumscribe morphology, topography and developmental changes of anatomical structures in the infrahyoid triangles of human neck during prenatal period of human development and provide morphometrical data of those.

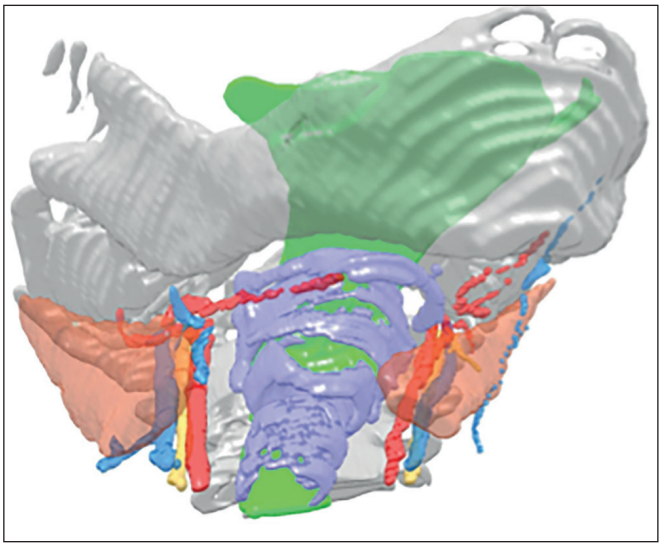

Fig. 1. 3D reconstruction of anatomical structures of the anterior triangle of neck of human prefetal specimen 55,0 mm PCL (10th week). Antero-inferior view. Magn.: x12: 1 – mandible; 2 – sternocleidomastoid muscle; 3 – hyoid bone; 4 – main vasculo-nervous bundle of the neck; 4.1 – common carotid artery; 4.2 – internal jugular vein; 4.3 – vagus nerve; 5 – trachea; 6 – thyroid cartilage; 7 – esophagus; 8 – vertebrae; 9 – external jugular vein.

MATERIALS AND METHODS

The study was performed in accordance with the provisions of the declaration of Helsinki (1995) as revised in Edinburgh (2000), ICH GCP (1996) and had been approved by the Bukovinian State Medical University Ethics Committee. Study is a part of complex scientific work of the Department of Histology, Cytology and Embryology "Regularities of morphogenesis and structural features of tissues and organs in human ontogenesis" (registration number 0116U002938).

Specimens of the fetuses (of both genders) from the Chernivtsy Regional Pathologists Office had been used for research in accordance to bilateral contract on scientific collaboration with the Department of Histology, Cytology and Embryology.

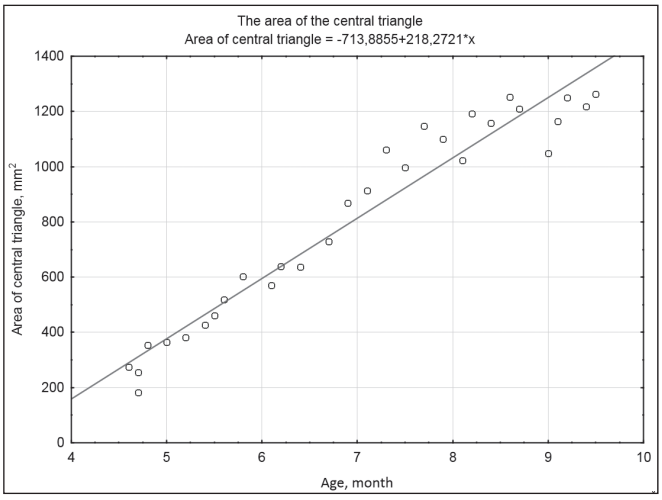

Fig. 3. The area of the central triangle in human fetuses 4th – 10th month of intrauterine development (85,0-360,0 mm PCL).

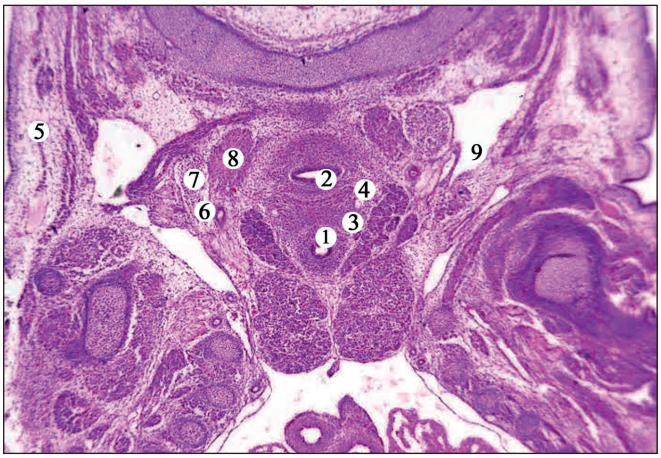

Fig. 2. Frontal section of the neck organs in the human prefetus 30,0 mm PCL. Stained with H&E. Magn.: x30: 1 – trachea; 2 – oesophagus; 3 - retropharyngeal space; 4 - recurrent laryngeal nerve; 5 – platysma; 6 - common carotid artery; 7 – sternocleidomastoid; 8 – sternothyroid; 9 – omohyoid.

The specimens of human prefetuses aged from 7th till 12th week (16,0-82,0 mm of parieto-coccygeal length ((PCL) and human fetuses aged from 4th till 10th month (84,0-360,0 mm PCL) of intrauterine development (IUD) were investigated by means of morphological methods (macroscopy for fetuses, microscopy for prefetuses (with fixation, embedding in dense material, staining with haematolixil & eosin), 3D-reconstruction for prefetuses (based on series of histological labeled slides, n = ± 50 from each prefetus specimen), morphometry and statistical analyses). All specimens were obtained from ectopic pregnancies or spontaneous abortions, and no part of the material gave

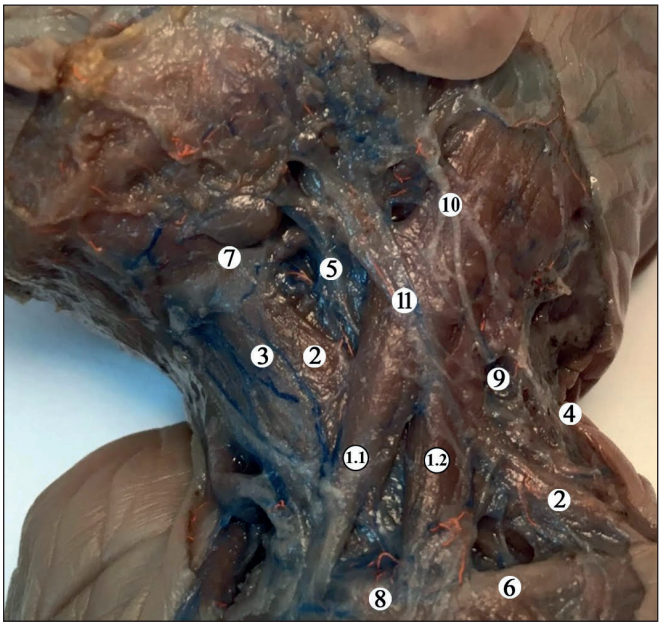

Fig.4. Borders of infrahyoid triangles. Macrospecimen of male human fetus 150,0 mm PCL, left view. Magn.: x2: 1 – sternocleidomastoid muscle and region: 1.1 – sternal head, 1.2 – clavicular head; 2 – omohyoid muscle; 3 – sternohyoid muscle; 4 – trapezius muscle; 5 – braches of v. jugularis interna; 6 – clavicle; 7 – hyoid bone; 8 – supraclavicular nerve twigs; 9 – twigs of cervical plexus.

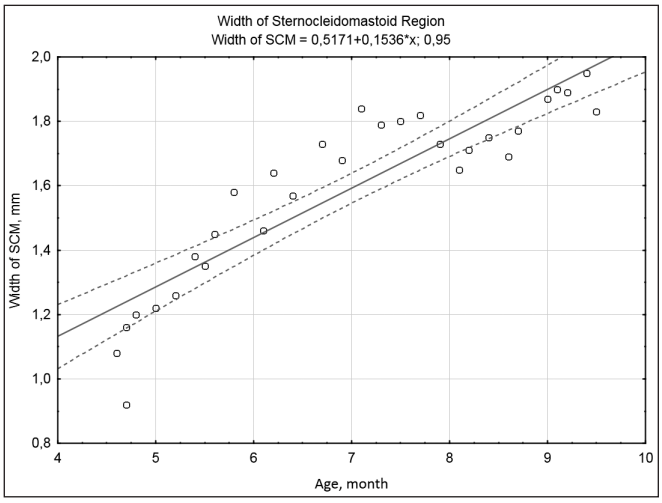

Fig. 5. The width of sternocleidomastoid region in human fetuses 4th-10th month of intrauterine development (85,0-360,0 mm PCL).

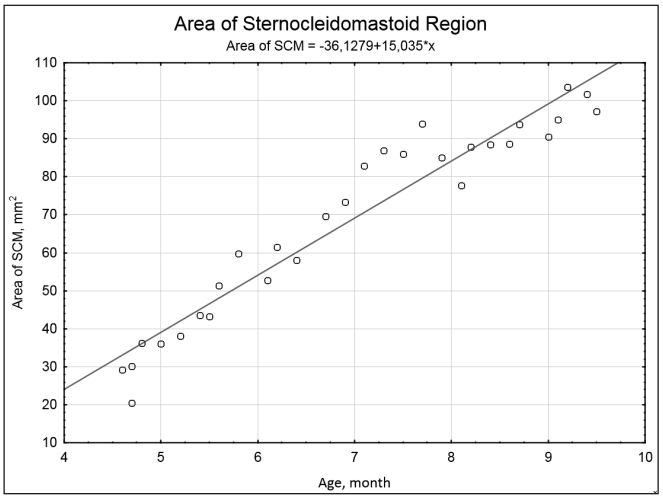

Fig. 6. The area of sternocleidomastoid region in human fetuses 4th-10th month of intrauterine development (85,0-360,0 mm PCL).

indications of possible malformation. Series of histological specimens (embedded in paraffin and stained with haematoxilin and eosin) from prefetal material were conducted by microtome in sagittal and horizontal axes with photographing. Micro- and macroscopy, morphometry was conducted under the control of binocular microscope.

RESULTS AND DISCUSSION

We have observed that the infrahyoid portion of the neck is represented by triangular areas that are found in space between the hyoid bone (HB) downwards to thoracic inlet starting from 55,0-60,0 mm PCL prefetuses and later on in the fetal period of the IUD. IH region (including infrahyoid triangles) are seen on three-dimensional reconstructions in middle-aged human prefetuses (35,0-65,0 mm PCL) by the means of outlined IH group of muscles, main vascular structures and by the main landmark for delimiting infrahyoid region – the HB (Fig. 1). Three-dimensional reconstruction is believed to be a reliable method for early human development studies [11, 12] and we have used it to investigate topographical peculiarities in human fetus o f10th week developmet.

With the help of histological investigation of prefetal material and macroscopic studies of fetal specimens, we have seen that spaces in the infrahyoid region are divided by the means of the deep cervical fascia leaves. Visceral, carotid and the prevertebral spaces are seen much clear comparing to retropharyngeal and posterior cervical in the prefetal period (30,0-80,0 mm PCL). The superficial covering for infrahyoid neck is skin, superficial neck fascia and platysma. Platysma is represented by a broad thin line of muscular fibers going in one direction, placed immediately beneath the hypodermis. Platysma in fetuses originates from the clavicle and acromion and has oblique direction of fibers towards its insertion to the mandible near the external oblique line; in prefetuses it has shown bigger content of fibrous component comparing to those in fetuses.

We can observe anterior triangle in human fetuses after the time when common precursor muscular mass splits into two: the anterior and posterior portions which will give rise to the sternocleidomastoid (SCM) and trapezoid muscles accordingly (Fig. 2). This period is important as a possible time for congenital malformations development [13, 14].

This histological picture may be found starting from the middle prefetal period (40,0 mm PCL specimens and on), when SCM has enlarged to the definitive shape and is fixed at its anatomical attachment points (mastoid process in superior portion and the clavicle in the inferior portion of the neck). As a result of this split we can observe boundaries of the anterior triangles in human neck and those lined above by the precursor of HB will be the infrahyoid ones: carotid, omotracheal, omoclavicular, omotrapezoid and SCM region, that are often used in reconstructive surgeries [8, 9]. The mandible superiorly, anterior borders of SCM laterally and the midline of neck are the margins for the AT, covered by the superficial layer of the deep neck fascia and supported deeply by the visceral layer of the deep neck fascia. Multilayered configuration of the neck can be seen starting from 90,0 mm PCL in fetal period, as we have seen that deep cervical fascia has developed its subdivisions: superficial, visceral and deep layers. Fascial layers create fascial spaces, that can be distinguished macroscopically starting from early stages of fetal period (85,0-100,0 mm PCL and on) as they develop some volume of the fatty tissue inside and later on can be classified using an estimated topographical classification [15].

The area of AT is important index for us as it generalizes the content volume of the strap-like infrahyoid muscles: omohyoid (OH), sternohyoid (SH), thyrohyoid, sternothyroid (ST). Blood supply of IH muscles in fetal period (7^{th}-10^{th} months) is supported by superior and inferior thyroid arteries. The area index (mm^2) of the central triangle (-713,8855+218,2721*x) in human fetuses 4^{th} – 10^{th} month of IUD (85,0-360,0 mm PCL) shows the increasing tendency during IUD with the highest rates at 8^{th}–10^{th} months period – 1100-1200 mm^2 (Fig. 3).

Moreover, Figure 3 shows the general dependence of fetal age (in month of IUD) to the area of anterior triangle and we may deduce the deviations of indexes during each month which shows high rate of individual anatomical distinctions. Even

though this area index reaches its peak point around 8,5-9th months of IUD, the critical period of its morphogenesis should be considered 7th month of IUD. This can be explained by the highest intensity of bony structures development (mandible, clavicle, sternum) as well as the critical period for muscular, vascular and fascial structures IUD modification.

Muscular triangle (MT) is lined anteriorly by the midline of neck from the HB to the sternum; posteriorly by anterior border of SCM; apically by superior belly of OH muscle. At middle of prefetal stage (3rd month of IUD; 54,0-80,0 PCL) OH muscle is found to be innervated from the twigs on ansa cervicalis (AC) and seen as a straight long muscle without intermediate tendon. The angulated course of OH is visible at late prefetal and early fetal periods (3-4th month; 80,0-130,0 PCL) as well as the presence of intermediate tendon. OH anterior belly arises behind the SCM as a continuation of the posterior belly, ascending almost vertically in close topographical relation to SH muscle (Fig. 4) and attaches to the lower part of HB, that correlates with anatomical studies on OH morphology [16, 17].

The roof of MT is represented by skin, superficial fascia, thin platysma (innervated by superficial braches of the cervical plexus) and leaf of deep neck fascia. Floor of MT is represented by SH and ST muscles. SH and ST are found to be covered by deep layer of the deep neck fascia that is seen as thick layer of dense connective tissue, attached to the posterior border of the HB. In late fetal period (230,0-350,0 PCL; 7-9th month of IUD) the space between two layers of the deep cervical fascia was found to contain moderate amount of adipose tissue. MT contains common carotid artery (CCA), IJV and vagus nerve that lies between the vessels (Fig.4).

SCM runs obliquely across each sides of the neck and is covered by two layers of deep cervical fascia. Prefetuses 55,0-80,0 PCL already show the presence of two heads in the place of SCM attachment to the sternum and clavicle (Fig.4). Sternal head of SCM starts as a tendon at upper anterior portion of the sternum, that goes upwards as a dense muscle fiber turning backwards obliquely. Clavicular head of SCM is attached to the inner 1/3 posterior border of clavicle as a compact muscle fiber directed vertically upwards. The space between the arising heads of the SCM varies in its triangular shape and size depending on the total lengths of the SCM and its width. Both heads fuse together to form a SCM at the level of HB and attach to the mastoid process. The width of SCM region (Fig. 5) has evaluated gradually during fetal period (width of SCM = 0,5171+0,1536*x; 0,95) that may be used while studying peal force capabilities of the SCM [18].

Critical periods of its formation may be considered 6th and 7th months of IUD (190,0-260,0 mm PCL). Deviations of indicators from Fig. 5 can prove the variety of morphological course of the SCM development: width can predict the area index and the morphological features of topographical neighboring structures in the IH neck of the future newborn. Deviations of abovementioned index 9if measured prenatally) can be predictor for abnormal course of the SCM morphogenesis (wryneck, development of additional heads etc. [18]).

Area index of the SCM region (Fig. 6) that was analyzed statistically (area of SCM = -36,1279+15,035*x), shows that the highest parameter can be found at the 9th month of IUD. This index varies and each IUD period, but also tends to increase gradually. We have observed the highest rate (peaks of evaluation) in 190,0-210,0 mm PCL and 260,0-270,0 mm PCL human fetuses, 6th and 7,5th months accordingly (Fig. 6).

Area index of SCM that we have measured can be used clinically for the prenatal diagnostic, as the SCM region itself holds part of CCA, IJV and vagus nerve. IH portion of SCM in fetal period is supplied by branch of superior thyroid artery.

SCM is the posterior border for the carotid triangle, that contains upper part of CCA and is a place of its bifurcation on external (ECA) and internal carotid arteries (ICA); hypoglossal nerve and the accessory nerve. In the medial portion of ECA, close to HB we have seen internal branch of superior laryngeal nerve. The inferior border for carotid triangle is the superior belly of the omohyoid; superior border is held by stylohyoid and posterior belly of the digastricus. Roof in carotid triangle is represented by skin, superficial fascia, platysma, investing layer of the deep cervical fascia. CCA runs cranially and complements neurovascular bundle of the neck. Human prefetuses (82,0 mm of PCL) show the presence of fascial coverings (sheath) of the carotid space that are formed as prolongations of the deep cervical fascia covering. We have found fibrous septs between each of the blood vessel within cervical vascular trunk which were included in one common covering. On the right plate of the infrahyoid region of prefetal neck (70,0-82,0 mm PCL), right CCA lies between trachea and anterior scalene muscles. AC lies in the anterior wall of carotid layer which is overlapped by anterior edge of SCM. Terminal branches of AC in prefetal and fetal periods show the variety of topographical anastomoses that supply innervation of the infrahyoid muscles [19]. Behind the carotid vessels we may dissect prevertebral fascia that participates in creation of carotid sheath. In front of the carotid vessels next to investing layer of neck fascia we may see pretracheal fascia that continues to the HB.

We haven't found any signs of branchial structures residuals at the prefetal stage (20,0-80,0 mm of PCL) that is why no possible congenital abnormalities were observed. We also have not found the residuals of thyroglossal duct at this stage within the strap muscles which means that at prefetal stage tissue of thyroid lobules has isolated from pharyngeal precursor. The thyroid cartilages (cricoid, thyroid and arytenoids) are fully developed in prefetuses 67,0-80,0 mm PCL and finishes the ventral fusion in early fetal period (96,0 mm PCL). Two laminae of the thyroid are formed and we may see ventral fusion also.

The vertebral vein is found to descend above the foramen magnum following the course of the posterior jugular vein in prefetuses. It runs above the vertebral artery and descends vertically in the canal of the transverse process of cervical vertebrae next to the artery. Running above the subclavian artery it ends in a trunk to subclavian jugular confluence. The vertebral vein drains venous blood from the deep structures of the IH region and deep cervical veins. Posterior jugular vein is found to be connected with IJV and vertebral veins. IJV together with subclavian veins create jugular venous angle, which may be considered as the content of lateral triangle of neck and should be carefully operated while conducting neck infection surgeries [2], especially in cases of additional infrahyoid muscles presence [20].

CONCLUSIONS

1. Prefetal period of human ontogenesis is characterized by intensive processes of organogenesis and formation of infrahyoid neck triangles with district borders and content.
2. Fetal period of prenatal development (4^{th}-10^{th} months of IUD) shows definite morphology of infrahyoid neck that is close to an adult one: muscular, carotid and SCM regions are differentiated with fully developed vascular, muscular and fascial content.
3. Morphometrical indexes of IH neck rely on topographically close structures. The critical periods for the AT and SCM regions should be considered 6^{th} and 8^{th} months of the IUD. Provided morphometric data may be used as some prenatal diagnostic criteria for human fetuses of last trimester.

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ORIGINAL ARTICLE

PREVENTING THE PRE-SICK CONDITIONS OF THOSE WHO PRACTICE LIFELONG LEARNING

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ABSTRACT

The aim: Disclosure of the results of the study of the expediency of considering the fluidity of mental processes in the triad "organism – personality Disclosure of the results of the study of the expediency of considering the fluidity of mental processes in the triad "organism – personality – environment" and the alternate stages of the dynamics of performance to prevent the occurrence of pre-painful conditions in students, combining professional activity with formal education.

Materials and methods: The complex of research methods is used in the work: general scientific (analysis, synthesis, comparison, systematization, generalization) and empirical (observations, discussions, questionnaires). The research was carried out within the framework of the international project "AHIA" (innovation in education; access mode: https://sites.google.com/view/project-axia/). The Spielberger-Hanin questionnaire was used to achieve the stated purpose of the study, the purpose of which was to assess reactive and personal anxiety.

Results: The results of a study aimed at organizing a lifelong learning process based on mental processes and stages of performance dynamics showed that the likelihood of pre-painful conditions in those who combine full-time education in higher education with professional activity significantly decreases their self-actualization. Educational events taking place here and now, and recognizing the theoretical and practical significance of the educational material at the level of intrinsic motives.

It is established that the likelihood of pre-morbid conditions in those who learn during life significantly increases in the case of reduced functional reserves of their body as a result of the intense and prolonged performance of educational activities with the simultaneous experience of "negatively" colored actual emotional states, generated by activities or events) experiences of relationships in professional activity, family, family, etc.

Conclusions: The proposed article describes the effectiveness and appropriateness of using mental processes and stages of performance dynamics as a means to prevent the onset of painful conditions in lifelong learners.

KEY WORDS: mental processes, dynamics of performance, stages of performance

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INTRODUCTION

Man is the carrier of the psyche, and his body is the carrier of life. This indivisible whole is not a static formation since a person is constantly in some mental state. According to S. Maximenko [1], mental states are always, they are different and always change each other, activating certain functional systems of the human body. Thus, the nature of psychological states is a constant dynamic, a movement that, if artificially stopped, immediately becomes another state.

Various interpretations of the phenomenon of "mental state" are given in scientific sources. These include:

- "holistic characterization of mental activity for a certain period of time, which shows the peculiarity of the course of mental processes, depending on the objects and phenomena of reality that are reflected, the previous state and mental properties of the individual" [2, p. 18];
- "the form of existence of individually-unique human psyche" [1, p. 461];
- "a set of mental characteristics that show the level of dynamic parameters of the psyche – emotional, cognitive, behavioral, which respectively affect the educational (professional) activity and behavior" [3, p. 51].

According to S. Maksimenko's generalizations [1], state:

- is a pervasive psychic phenomenon that colors all mental activity;
- is determined not only by the imprint but also by the previous mental state and future;
- is a true indicator of individuality because it not only broadcasts mental phenomena but also shapes them, in other words, mental states affect other mental phenomena, change, rebuild, rebuild them;
- generates thought, which means that the mental state, forming based on a certain thought inter-functional system, integrates it with the experience, properties, aspirations of man, and only then the thought becomes the thought of that person.

The peculiarity of mental states is that "the depth of penetration of states is not limited to the psychic sphere alone. A cross-functional system, which integrates one or another state, necessarily "enters" into the field of biology,

and therefore medicine, and thus the mental state seems to "close" the space of human existence "[1, p. 461]. For example, a scientist examines a situation where a person begins to become ill with the flu and develops a so-called pre-sick condition, which covers virtually all areas of the individual. These are the slowing down of cognitive processes, attenuation of interests and cognitive activity, the appearance of sluggishness and increased fatigue. This condition begins with the cellular level of the body, as this level is infected by a virus.

This pre-morbid condition can be caused not only by the interaction of the virus with the cell of the human body but also by the result of performing activities (educational or professional) with a high and prolonged level of tension, which is caused by the need to perform actions in conditions of time and information deficit; a significant amount of work assigned to the work; awareness of the high responsibility for the result of one's own actions, as this affects, for example, the passing of a test, an exam, obtaining a scholarship, diploma, salary, position, etc.; monotonous or dynamic mental loads; the excitement of being able to do the work at all, in the allotted time, according to requirements that are only partially understood; experiencing negative emotions from interacting with Others (classmates, teachers, colleagues, etc.).

According to the results of scientific researches O. Yeshchenko, V. Kalnysh, V. Yeshchenko [4] in the pre-morbid state a number of complaints characterizing the deterioration of the state of health is formed, which is manifested in increased irritability and unstable mood (in 25% of cases); feelings of depression, weakness (in 20% of cases) and fatigue (in 55% of cases). These factors lead to a decrease in the adaptability of the organism, in particular to a weakening of the respiratory system, a decrease in the resistance of the organism to the effects of adverse environmental factors, and for irritability to disorders in the functioning of the gastrointestinal tract, persistent and long-term decrease in appetite. Also, before the painful condition increases the level of fatigue, which, in turn, causes changes in the indicators of such psychophysiological characteristics as functional mobility, strength, and lability of nervous processes.

THE AIM

The purpose of this work is to substantiate the need for organizing a lifelong learning process with the observance of symmetry in the triad "organism – personality – environment", as well as focusing on the types of mental states that are necessary for the emergence of tumors in the structure of personality, without harming the health. those who combine professional activity with formal education.

Hypothesis of the study: the awareness of the person's mental states as a comprehensive mental phenomenon, showing the level of dynamic parameters of the psyche, namely: emotional, cognitive, behavioural, which, in turn, affect the professional and educational activities of man and his behaviour. The objectives of the study: 1) to generalize scientific data on the types of representation of mental states; 2) to establish the presence / absence of dependence between traits of a person's character and his / her long experience in certain mental states; 3) to analyse the stages of human performance with the emphasis on the dynamics of emotional, cognitive and behavioural parameters of the psyche; 4) summarize the results of the survey of the respondents regarding the factors that cause the manifestation of "negatively" colored actual emotional states; 5) to formulate conclusions about the peculiarities of organizing the process of human learning throughout life in the context of preventing the occurrence of pre-morbid conditions.

MATERIALS AND METHODS

The complex of methods is used in the work: general scientific (theoretical analysis, synthesis, comparison, systematization, generalization) and empirical (observations, interviews, questionnaires). The survey involved 527 respondents combining full-time higher education with a professional background.

RESULTS AND DISCUSSION

We emphasize on the achievements of scientists who, in our opinion, are important for formulating conclusions about the possibility of preventing the occurrence of pre-morbid conditions during lifelong learning. During the study of scientific sources, it was found that various scientists have joined the attempt to classify mental states, while formulating their generalizations. Thus, M. Levitov [2] singled out: 1) states of personality (in which, first of all, individual features of a person are expressed) and situational (features of situations that often cause a person uncharacteristic reactions to it); 2) states are deeper (for example, passion) and more superficial (such as mood); 3) states that have a positive effect on humans (in particular, inspiration) and states that negatively affect them (eg, apathy); 4) the states are short-lived (from a few minutes) and long (from days to several days); 5) states that are less aware (such as diffusion) and states that are more conscious (such as determination; fatigue; it may have different degrees of awareness).

L. Kulikov [5] classified mental states into short-term and long-term by temporal parameter and by emotional, activation, tonic, and tensile by leading parameter. In turn, the scientist ordered the emotional states according to the modality of the corresponding emotions, the activation states – by the level of motivation and completeness of inclusion in the situation, tonic states – by the level of the general tone of the organism related to human health, and tense states – by the degree mental and psychophysical tension of the body.

M. Korolchuk, V. Krainiuk, V. Marchenko [3] divided mental states into simple (depression, joy) and complex (adaptability, monotony, fatigue), and also formed couples based on a corresponding combination of positive mental states with negative, as- here, confidence is uncertainty;

cheerfulness – irritability; exaltation – depression; calm – affect; exaltation – stress; readiness for action is a psychological shock.

In the work of K.-G. Jung is talking about a chronic condition. According to the psychologist, if in some way the condition becomes chronic, the consequence of this is the emergence of a type, that is, a habitual setting in which one mechanism is constantly dominant, although it cannot completely suppress the other, since it necessarily belongs to the mental activity of life. [6, p. 123].

In the context of the occurrence of pre-morbid conditions during lifelong learning, the attention of scientists to the process of finding a person in one or another chronic condition, which in this case correlates with the long-term state, draws attention. According to S. Maksimenko, any trait (whatever the sphere of the mental it is related to) is in one way or another an embodiment of the habitual and lasting condition of a person. The scientist also analyzes the feedback, which is manifested in the fact that character traits and personality traits largely determine what states and how a person will experience. According to S. Maksimenko [1], it makes sense to speak about a person's inclination to certain states, about the individual internal picture of the flow of ETS states, as well as about the mediation of the mechanism of formation of new personal structures by the mental processes. Dynamic phenomena of the inner world of the individual, and above all, different types of intentions (the orientation of consciousness, to thinking on any subject), tend to be embodied (objectified) in the purpose, actions of the individual, and then in its properties. The generated state, as the embodiment of the personality orientation, forms a cross-functional system, and only after that "embodiment proceeds in this way. In this case, the mental state controls the whole process, and this control is relevant, that is, corresponding to this individuality.

According to O. Prokhorov [7], the dynamics of correlation of mental states and mental neoplasms of personality are significant. For its disclosure, the scientist distinguishes two types of states: the state of equilibrium and the state of disequilibrium. The reference points are the states of relative equilibrium (states of average or optimal mental activity), which may include states of rest, empathy, focus, mental adaptation, interest, etc. Conditions associated with the increased mental activity (joy, delight, anxiety, etc.), as well as states of reduced mental activity (delirium, depression, fatigue, sadness, etc.), which are characterized by a correspondingly higher or lower level of activity, attributed to scientists states of disequilibrium. These states occur when symmetry is violated in the triad "organism - personality - environment" [8, p. 84]. A new functional system (state) is formed, which is characterized by certain stress and uncomfortable experiences. Having a certain excess of energy, a new (asymmetric) cross-functional system generates an orientation of consciousness, thinking of a person on any object (in other words intentions), causes peculiar actions and actions, and, in the end, leads to the emergence of new tumours of personality. An important feature of the state of disequilibrium is to be a link in the process of the emergence of mental tumours, and an important function of these states is "the conditioning of the process of emergence of tumours in the structure of personal properties" [7, p. 86].

The scientist notes that the deeper and more acute is the state of disequilibrium, the more active the process of emergence of tumours is, the more deep and essential structures the personality manifests. The progressive solution of the subject of the tense situation involves integrative processes of personality and self-development. That is, due to special activity and behaviour, new structures are formed from the mental state of the person.

Determinants of imbalance states may be "passion, the significance of the situation, the allocation of the most essential circumstance in it, the factor that brings the most disorganizing beginning" [7, p. 89], as well as "a regular change in the stages of age development, age crises, development of the disease, the possibility of deployment along with the progressive and regressive path of solution-embodiment of states of imbalance" [1, p. 463].

In the context of this article, the regressive unfolding of disequilibrium states is regarded as a highly probable marker of the emergence of pre-morbid states, which in turn cause psychophysiological peculiarities of changes in performance, growing sense of fatigue, development of fatigue, as a result of intense or prolonged activity, lead to deterioration of quantitative and qualitative indicators of work, accompanied by a decrease in functional reserves of the body, as well as characterized by discoordination of physiological functions and increased physiological cost of work" [8, p. 270]), chronic fatigue (" a set of stable morphofunctional changes that slowly accumulate in the human body over a long period (months, years) the result of her work and are characterized by the gradual exhaustion of functional reserves and the presence of stable systemic and non-specific innately of mental and physiological shifts) [8, p. 270]) and appear to tend to change health.

It is advisable to consider the unfolding of the equilibrium states in the context of the dynamics of labour, which, per the studies of F. Kosmolinsky and E. Derevyanka [9], reflects the relationship between the functions of the organism, labour productivity and the development of fatigue against the background of emotional and volitional stress considering maximum capacity of the body. In turn, the productivity of the lifelong learning process is determined by the maximum capacity of the organism to effectively combine work with educational work, emotional and volitional tension and depends on the individual characteristics of the human body, its attitude to labour and educational work, a sense of responsibility and conditions of exercise learning activities and their combination to implement a lifelong learning process.

Performance dynamics are observed for events of varying duration, including short-term (one work and (or) school day) and long-term (semester, school year, contract term) and includes five stages (entry into work, relative to became able to work, lost working capacity as a norm of working

capacity, gradual decrease in working capacity, ultimate impulse) [3, p. 177]), which are considered separate functional states.

We analyze the stages of performance dynamics taking into account the process of human learning throughout life, that is, because of the combination of work activity with educational activity in formal education. The preparatory stage is characterized by the state of switching from one activity to another (for example, from professional activity to educational). Although the corresponding actions of the educational activity have not yet begun, the human body, which is included in the organized learning process, is already preparing for mental load, its functional capabilities are mobilized, and the vegetative processes that provide the energy of the organism are enhanced. As a result, working capacity increases even before the beginning of educational work. This is how scientists describe this stage [9; 10; 11], however, the analysis of empirical data showed that at the preparatory stage, students will experience the manifestation of "negatively colored" actual emotional states caused by various factors, including feeling anxious: unpreparedness to study (seminar, practical, modular) control, etc.) (in 5% of cases); expectations of unwanted emotional distress from future communication with some other participants in the educational process (with certain classmates or (and) teachers) (in 9% of cases); from the content of the educational material, which is subjectively perceived with a high level of psycho-emotional stress (in 16% of cases); professional events (in 25% of cases); dominant mood in the professional community (in 2% of cases) or born based on communication with parents of students (in 7% of cases); attitude towards colleagues, parents of students, members of their family (family) (in 33% of cases); test results of the students' control work (in 3% of cases).

The stage of entry into the activity, in our case in the educational, is characterized [9; 10; 11] gradual increase in the functional capacity of the organism, improving the productivity of activities with the simultaneous adaptation of the person to the most economical mode of performance. The analysis of empirical data showed that the duration of this stage depends on internal (in 91% of cases) and external motivation of students (in 72% of cases); the intensity of their mental actions (in 78% of cases); the impact of adverse factors on the body (in 63% of cases); increasing feeling of fatigue (in 99% of cases); continuation of the experience of emotional stress caused by previous events in professional activity, family life, social environment (in 14% of cases) and so on.

At the stage of unstable performance as the norm of performance is fixed [9; 10; 11] decrease in the maximum capacity of the body due to the appearance of the first signs of fatigue and its subjective manifestation - fatigue. Labor efficiency remains at the previous level due to the emotional-volitional stress and the use of compensatory mechanisms of the organism, in the case of the motivation of the learner, to cognitive activity in the learning process. According to the empirical data, at this stage, there is a need to increase the time required to carry out actions in the educational activity (in 78% of cases) and to return to the uncomfortable experiences caused by previous events in professional activity, family life (in 14% of cases).

Stages of gradual decline in performance are inherent [9; 10; 11] the processes of further development of fatigue and the appearance of fatigue. According to empirical data, mistakes are made in 32% of cases when performing even simple tasks.

The final gust stage takes place [9; 10; 11] at the end of the learning activity if the time of its completion is clearly defined. The level of performance increases due to the emotional-volitional stress and the use of physiological reserves of the body. The feeling of fatigue seems to disappear to manifest itself in a more pronounced form upon completion of the educational activity. Instead, the analysis of empirical data showed that the level of performance at the final impulse stage can be significantly reduced for informing learners the task for self-fulfilment, which is defined as difficult (in 45% of cases), voluminous (in 89% cases), uninteresting (in 78% of cases), has no practical significance (in 93% of cases). In this case, the feeling of fatigue is exacerbated by the experience of frustration and depression. Expansion of the state of imbalance becomes regressive since the functional reserves of the body are used not to form mental tumours, but to overcome the information stress caused (in this case) by information overload, and to experience the state of excitement caused by the unmet need for self-affirmation.

According to the purpose of the study, it was established that mental states should be considered as a form of existence of the individual-unique psyche of a person; an all-encompassing psychic phenomenon that colors all of its mental activity; a valid indicator of individuality. It should be borne in mind that mental states are constantly changing, activating at the cellular level different functional systems of the body and nervous processes, different psychophysiological characteristics (functional mobility, strength, lability). The originality of the study is that the prevention of pre-morbid conditions in life-long learners is considered at the level of mental states and stages of performance dynamics. The question of the impact of the results of human mastery on self-management techniques on the progressive/regressive decision-embodiment of imbalance states and the impact of these states on human health remains debatable.

CONCLUSIONS

Based on the analysis of scientific sources, it has been found that mental states, being "a form of existence of an individually-unique human psyche" (according to S. Maximenko), not only show mental phenomenon but also shape, modify, reconstruct and rebuild them. The effect of mental states manifested at the cellular level of the body, is the subject of research in various fields of science, in particular in psychology, biology, medicine, didactics.

Mental states may be states of personality / situational, deeper / more superficial, positive/negative effects on

humans, short-term / long-term, simple/complex, less conscious / more aware, emotional, invigorating, tonic, tense, chronic, pre-morbid also as states of equilibrium (states of rest, empathy, focus, mental adaptation, interest, etc.) and states of imbalance (these states are characterized by higher (joy, delight, anxiety, etc.) or low (depression, fatigue, sadness, etc.) ness.

In states of disequilibrium that arise when symmetry is violated in the triad "organism – personality – environment" (according to O. Prokhorov), a new functional system (state) is formed, characterized by a certain tension and uncomfortable experiences. With a certain excess of energy, a new (asymmetric) cross-functional system generates the orientation of the consciousness (thinking) of a person on any subject, causes certain actions and actions, which in the case of the motivation of the learner, leads to interest in the content of the learning process, and as a consequence. The gradual emergence in the psyche of new personal formations. However, this process does not take place without motivation through the learning process or its content. Under such conditions, it is most likely that pre-morbid conditions may occur that result in a significant decrease in the functional reserves of the human body as a result of the strenuous and prolonged performance of training actions with the simultaneous experience of "negatively"colored actual emotional states, generated by events during the training event this (in our case it is a professional activity), or experiences of events (relationships) that relate to the family etc.

When organizing the process of lifelong learning, one should also consider the dynamics of performance, which is manifested in a successive change of such five stages as 1) getting into work; 2) relatively able to work; 3) inefficiency as a norm of efficiency; 4) gradual decline in performance; 5) ultimate impulse. These stages take place in an educational process of varying lengths, in particular during one training session (lectures, seminars, etc.), as well as during one academic day, one semester, one academic year or a period of study in a higher education institution.

Organization of a lifelong learning process, taking into account the mental processes and stages of the workability dynamics, to prevent the occurrence of pre-morbid conditions in the learners, since the self-realization in the real educational events that take place here and now, their current and meaning, will have significant meaning and significance will generally be consistent with internal motives.

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ORIGINAL ARTICLE

MORPHOLOGICAL EFFICACY EVALUATION OF GEL WITH CARBON DIOXIDE EXTRACT OF HOPS IN CASE OF COMPLICATED WOUND INFECTION ACNE VULGARIS

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ABSTRACT

The aim of the study is to reveal in the experiment the morphological features of the infected skin wounds healing, which are a manifestation of acne vulgaris severe and very severe forms, using a gel with carbon dioxide extract of hops.

Materials and methods: An experimental study was carried out on 80 male WAG rats of three months of age. The animals were divided into 9 groups. Group 1 consisted of intact animals (n=6). Group 2 was represented by animals (n=6), which had hair epilation on the dorsal surface of the body in an area of 1 cm^2, followed by application of 2 ml of placebo gel to this area. Group 3 included animals (n=6), which were epilated on the dorsal surface of the body in an area of 1 cm^2 and applied 2 ml of 1 % gel with carbon dioxide extract of hops. Group 4 included rats (n=6), which were epilated on the dorsal surface of the body in an area of 1 cm^2 and simulated thermal damage. Group 5 was represented by 10 rats, who were epilated on the dorsal surface of the body in an area of 1 cm^2, simulated thermal damage, followed by applying 2 ml of placebo gel to the wound surface. Group 6 included rats (n=10), who underwent measures similar to group 5, followed by application of 2 ml of 1 % gel with carbon dioxide extract of hops to the wound surface. In group 7, there were 6 rats, which were epilated on the dorsal surface of the body in an area of 1 cm^2, thermal damage to the skin with underlying soft tissues was simulated, followed by application to the wound surface the reference strains of *Staphylococcus aureus, Streptococcus pyogenes, Proteus vulgaris, Propionibacterium acnes, Escherichia coli, Pseudomonas aeruginosa, Malassezia slooffiae, Malassezia pachydermatis, Candida albicans, Candida parapsilosis*. In groups 8 and 9, there were 15 rats each, which underwent measures similar to group 7, followed by applying 2 ml of placebo gel on its surface on the next day after infection of the wound in group 8, and in group 9 – 2 ml of 1 % gel with carbon dioxide extract hops. The material for the study was the skin with underlying soft tissues. It was used histological, histochemical, morphometric and statistical methods.

Results: This experimentally created gel with carbon dioxide extract of hops activates separation processes of horny masses from the surface of the epidermis, cleaning the pores of the skin; stimulating the proliferative activity of the epidermis, which is located in the marginal sections of the wound or covers the surface of the regenerate; activating the processes of cleansing the wound from necrotic tissue; activating the growth and maturation of granulation tissue with its subsequent transformation into connective tissue. It has anti-inflammatory, bactericidal and antimycotic effects, normalizing skin microbiocenosis.

Conclusions: The complex morphological study has showed that gel with carbon dioxide extract of hops is a highly effective drug in treatment of severe and very severe acne vulgaris, characterized by the development of deep and infected wound defects.

KEY WORDS: morphology, complicated wound infection acne vulgaris, experiment, gel with carbon dioxide extract of hops

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INTRODUCTION

Acne vulgaris is a widespread polyetiological disease of the skin (sebaceous glands and hair follicles) with a complex multifactorial development mechanism which has a pronounced effect on the psychosocial sphere and social adaptation of patients [1]. Acne ranks first in the structure of cosmetology pathology and third in the frequency of patients' referral to dermatological medical institutions [2].

Acne vulgaris manifests during puberty and worsens throughout adolescence. Epidemiological studies suggest that it can arise at any age [3]. The study of the Global Burden of Disease reported that acne vulgaris affects about 85 % of young adults aged 12-25 years [4].

Acne, according to modern concepts, is considered as an inflammatory disease with a chronic recurrent course, characterized by hyperplasia of the sebaceous glands, hyperkeratosis and the formation of comedones – sebaceous-horny plugs in the ducts of the sebaceous glands with partial or complete blockage, as well as increased production of altered sebum composition [1].

Table 1. Components of the placebo gel and their ratio (weight percent)

Component	Component ratio in weight percent
Ethanol 96 %	2.500-7.500
Propyleneglycol	5.000-15.000
Polyethyleneglycol 400	5.000-15.000
Carbomer (carbopol)	0.600-0.800
Sodium hydroxide	0.070-0.120
Trilon B	0.025-0.075
Purified water	Remainder

Table 2. Components of 1 % gel with carbon dioxide extract of hops and their ratio (weight percent)

Component	Component ratio in weight percent
Carbon dioxide hop extract	0.500-1,500
Ethanol 96 %	2.500-7.500
Propyleneglycol	5.000-15.000
Polyethyleneglycol 400	5.000-15.000
Carbomer (carbopol)	0.600-0.800
Sodium hydroxide	0.070-0.120
Trilon B	0.025-0.075
Purified water	Remainder

Research has shown that inflammation precedes the formation of comedones and hyperkeratinization. Inflammation in the sebaceous hair follicle occurs even before the formation of rash elements and is manifested by a significant increase in the number of T-lymphocytes, macrophages, expression of interleukin 1 and alpha integrins. Interestingly, inflammatory changes are noted both in the place of subsequent formation of acne elements and in areas of healthy skin [5].

The pathogenetic factors of acne also include changes in the hormonal and immune status of the body, disorders of skin microbiocenosis [6, 7].

Studies have shown that the development and course of acne depends on a family (genetic) predisposition. There is a general pattern: the more often and more severe acne occurs in previous relatives, the more severe and torpid course of the disease will be in the next generation [8, 9].

Acne vulgaris is characterized by clinical diversity. According to the classification of the American Academy of Dermatovenereology, mild, moderate, severe and very severe degrees of severity of the disease are distinguished [10]. Also, acne is a chronic dermatosis, sometimes developing long-term non-healing wound defects and post-acne skin changes [2].

Despite the variety of approaches to acne vulgaris treatment with therapeutic and cosmetic means, this problem has not been completely solved [8]. P. Gibrad called acne one of the cornerstones, on which the efforts of physiologists, therapists and dermatologists are broken in the search for effective methods of treatment and explanation of the incomprehensible aspects of this disease [11]. Thus, the search for effective methods of treating this pathology remains an urgent issue for the medical community, which requires comprehensive clinical and experimental research.

THE AIM

The aim of the study is to reveal in the experiment the morphological features of the infected skin wounds healing, which are a manifestation of acne vulgaris severe and very severe forms, using a gel with carbon dioxide extract of hops.

MATERIALS AND METHODS

An experimental study was carried out on 80 male WAG rats of three months of age in the vivarium of the State Enterprise «Institute Microbiology and Immunology named by I.I. Mechnikov National Academy of Medical Sciences of Ukraine». The conditions of keeping and handling the animals were in accordance with the requirements of the «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasbourg, 1986).

The animals were divided into 9 groups. Group 1 consisted of intact animals (n=6). Group 2 was represented by animals (n=6), which had hair epilation on the dorsal surface of the body in an area of 1 cm^2, followed by application of 2 ml of placebo gel to this area. Group 3 included animals (n=6), which were epilated on the dorsal surface of the body in an area of 1 cm^2 and applied 2 ml of 1 % gel with carbon dioxide extract of hops. Group 4 included rats (n=6), which were epilated on the dorsal surface of the body in an area of 1 cm^2 and simulated thermal damage. Group 5 was represented by 10 rats, who were epilated on the dorsal surface of the body in an area of 1 cm^2, simulated thermal damage, followed by applying 2 ml of placebo gel to the wound surface. Group 6 included rats (n=10), who underwent measures similar to group 5, followed by application of 2 ml of 1 % gel with carbon dioxide extract of hops to the wound surface. In group 7, there were 6 rats, which were epilated on the dorsal surface of the body in an area of 1 cm^2, thermal damage to the skin with underlying soft tissues was simulated, followed by application to the wound surface the reference strains of *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (ATCC 6538-P), *Streptococcus pyogenes* (ATCC 19615), *Proteus vulgaris* (ATCC 4636), *Propionibacterium acnes* (ATCC 6919), *Propionibacterium acnes* (ATCC 11827), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Malassezia slooffiae* (CBS 7956), *Malassezia pachydermatis* (ATCC 14522), *Candida albicans* (ATCC 885-653), *Candida albicans* (Sklyar-31), *Candida albicans* (Sklyar-20), *Candida parapsilosis* (VKPGu 488/10). In groups 8 and 9, there were 15 rats each, which underwent measures similar to group 7, followed by applying 2 ml of placebo gel on its surface on the next day after infection of the wound in group 8, and in group 9 – 2 ml of 1% gel with carbon dioxide extract of hops.

Characteristics of the placebo gel and 1 % gel with carbon dioxide extract of hops are given in tables 1 and 2.

Thermal damage of the skin with underlying soft tissues, as a characteristic complication of severe forms of acne vulgaris, was modeled using a contact burn method. A metal plate heated to 90 °C was applied to a 1 cm^2 skin area epilated on the dorsal surface of the body for 10 seconds.

Animals of groups 1-9 were withdrawn from the experiment on day 30. The material for the study was the skin with underlying soft tissues. The material was fixed in 10% aqueous formalin solution on phosphate buffer (pH – 7.0-7.2). The compaction of tissues fixed in formalin was achieved by conducting through alcohols of increasing concentration, Nikiforov's liquid (96% alcohol and diethyl ether in a ratio of 1:1), chloroform and pouring into paraffin. Serial sections 4-5×10^{-6} m thick were made from the blocks for staining with hematoxylin and eosin, picrofuxin according to van Gizon, according to Mallory.

Microspecimens were studied, using an Olympus BX-41 microscope (Japan) with subsequent processing with the Olympus DP-soft version 3.1 software. A morphometric study was carried out during which the thickness of the stratum corneum, epidermis, leukocyte-necrotic layer, demarcation leukocyte shaft, granulation tissue and connective tissue was measured.

A statistical analysis was performed using the Statistica 6.0 and Microsoft Excel 2003 software. When comparing the parameters, the nonparametric Mann-Whitney U-test was used. The significance of differences between the mean values of indicators in the groups was taken at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

In survey microscopy in rats of group 1, the skin, consisting of the epidermis and dermis, with the underlying hypodermis and muscle tissue, had a normal structure with the absence of any general pathological processes. Eosinophilic keratin masses were revealed on the epidermis surface, which braided their shafts at the points of hair exit. The average thickness of the epidermis and the layer of stratum corneum was (20.11±0.69)×10^{-6} m and (6.07±0.13)×10^{-6} m, respectively.

In group 2, the skin with underlying hypodermis and muscle tissue had a structure similar to group 1. The average values of the epidermis thickness and the layer of stratum corneum did not differ significantly ($p>0.05$) from the indicators of group 1 and were, respectively, (19.79±0.52)×10^{-6} m and (5.99±0.24)×10^{-6} m. Our survey microscopy and morphometric study has showed that the placebo gel does not affect the morpho-functional state of the rat skin.

In rats of group 3, the skin with underlying soft tissues had a structure similar to that of group 1. In this group, the average value of the epithelial layer thickness did not differ significantly ($p>0.05$) from group 1 and amounted to (19.58±0.42)×10^{-6} m. The average value of the keratin masses layer thickness in this group was significant ($p<0.05$) lower value ((3.56±0.11)×10^{-6} m) compared to group 1. The decrease in the thickness of the keratin mass layer on the epidermis surface, revealed by us during the morphometric study in group 2, was due to the use of gel with carbon dioxide extract of hops, which, from our point of view, activates the separation of horny masses from the skin surface.

Exfoliation of horny masses is known to cleanse the skin of exogenous toxins, allergens and pathogenic microorganisms. Constant replacement of «dead scales» with «new scales» in the stratum corneum helps to maintain aggression of the external environment [12].

In group 4, a survey microscopy revealed a wound cavity, its depth reaching the deep parts of the dermis and hypodermis. The wound cavity was filled with mature granulation tissue, which was characterized by a pronounced predominance of the fibrous component over the cellular and vascular components (figure 1). The fibrous component was characterized by the presence of thickened fuchsinophilic connective tissue fibers with different directions, among which collagen fibers predominated over elastic ones when stained according to Mallory. The cellular component was represented by macrophages, lymphocytes, fibroblastic cells and leukocytes. The vascular component was characterized by vessels of various sizes. The average value of the granulation tissue layer thickness was (629.62±18.53)×10^{-6} m. Behind the layer of granulation tissue, a layer of connective tissue was revealed, which, when stained with picrofuchsin according to van Gieson, was in some fields of view light red, and in another fields of view dark red. When stained according to Mallory, collagen fibers predominated over elastic fibers in the connective tissue, and in some of the visual fields the latter were not detected. The average value of the connective tissue layer thickness was (134.29±11.08)×10^{-6} m.

There was an increase in the proliferative activity of the epidermis in the marginal parts of the wound, which thickened and formed superficial acanthotic ingrowths in some of the visual fields. In some of them a thickened epithelial layer crawled to the surface of the granulation tissue but complete epithelialization of the wound did not occur. The average values of the epithelial layer thickness and the layer of stratum corneum, covering the surface of the epidermis, were (22.12±0.67)×10^{-6} m and (6.14±0.52)×10^{-6} m, respectively. In this group in comparison with group 1, the mean value of the stratum corneum layer thickness did not differ significantly ($p>0.05$), and the mean thickness value of the epidermis significantly ($p<0.05$) increased, which, in our opinion, was due to the regenerative activation of the proliferative potential of epithelial cells.

In group 5 there were revealed similar to group 4 features of the layers in the wound cavity and the marginal epidermis of the wound. During morphometry, it was found that the average values of the thickness of the stratum corneum, epithelial layer, granulation tissue layer, as well as the layer of connective tissue were, respectively, (6.20±0.48)×10^{-6} m, (22.69±0.72)×10^{-6} m, (612.15±20.09)×10^{-6} m, (139.52±10.11)×10^{-6} m. Compared with group 1, the aver-

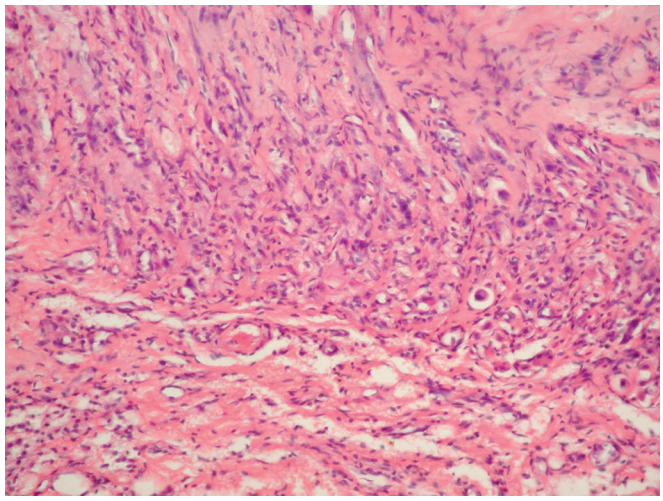

Fig. 1. Group 4. Mature granulation tissue, characterized by the predominance of the fibrous component over the cellular and vascular components. Stained with hematoxylin and eosin × 200.

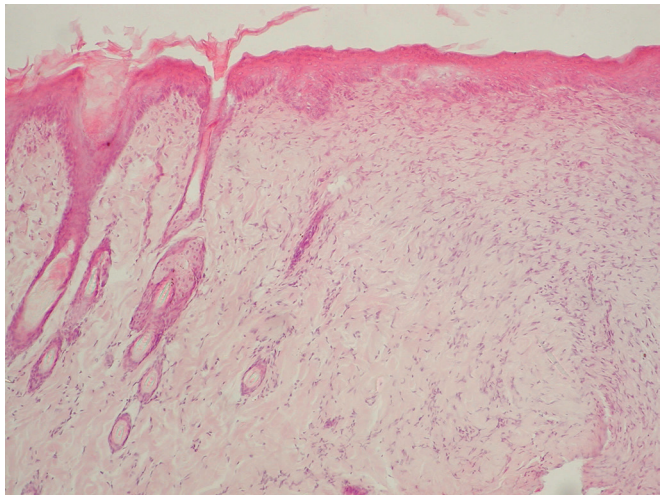

Fig. 3. Group 6. Formed regenerate with skin appendages. The surface of the regenerate is covered with thickened epidermis forming superficial and deep acanthotic ingrowths. Some horny masses on the surface of the epidermis. Stained with hematoxylin and eosin, × 100.

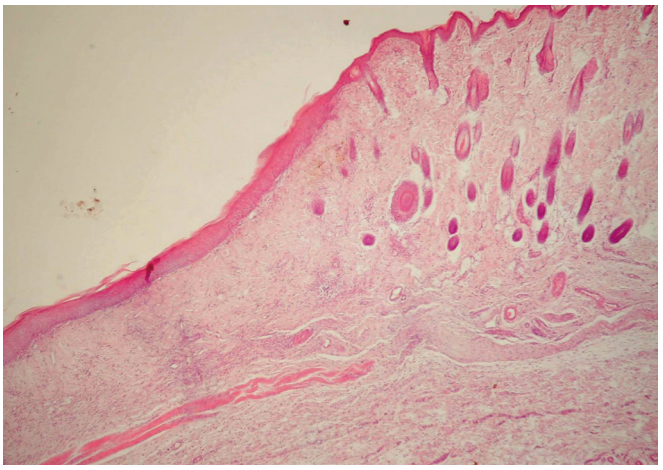

Fig. 2. Group 6. Atrophic skin scar. Some horny masses on the surface of the epidermis. Stained with hematoxylin and eosin, × 40.

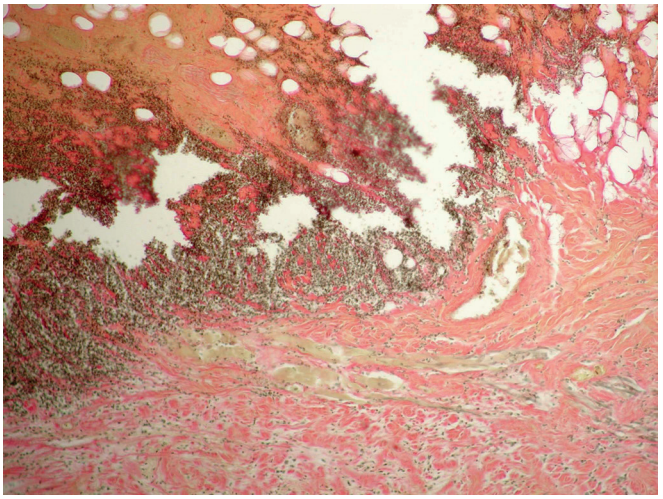

Fig. 4. Group 7. Leukocyte-necrotic layer, demarcation zone, layer of granulation tissue in the wound cavity. Van Gieson's picrofuchsin staining, × 100.

age thickness values of the stratum corneum and epidermis did not have significant ($p>0.05$) differences. In this group, compared with group 4, the average thickness values of a granulation tissue layer and connective tissue did not differ significantly ($p>0.05$). Thus, a comparative analysis of the results obtained in groups 5 and 4 has showed that placebo gel does not have any effect on the healing processes of the experimental wound.

The survey microscopy in group 6 has revealed complete healing of the simulated wound defect in all animals. A regenerate represented by connective tissue in 6 rats, was determined in the area of the modeled defect, skin appendages were not detected in it. When stained with picrofuchsin according to van Gieson, the connective tissue fibers were located in different directions, were thickened, dark red color. Among connective tissue fibers, when stained according to Mallory, a pronounced predominance of blue collagen fibers over red elastic fibers was determined, and the latter were absent in some of the visual fields. The surface of the regenerate was completely covered with a thickened epidermis.

It is interesting that in 2 rats of group 6 the connective tissue formed at the site of the wound defect, covered with the epidermis from the surface, was located below the surface of the surrounding skin, which corresponded to the structure of the atrophic skin scar (figure 2). In 4 rats, the formed connective tissue was located above the level of the surrounding skin surface, which indicated a hypertrophic scar formation.

In 4 rats of group 6, the connective tissue formed at the site of the wound defect was located at the level of intact skin, characterized by the presence of skin appendages, was covered with a thickened epidermis from the surface, which had a layer-by-layer organization, forming superficial and deep acanthotic ingrowths (figure 3). In the future, the latter will be the source of hair and gland buds formation [13]. The data obtained indicate the organotypic healing of the wound defect.

Organotypic regeneration, as is known, is one of regeneration types when an organ defect is replaced by a regenerate corresponding to the organ's structure [13, 14].

In group 6, the average thickness value of the epidermis layer covering the surface of the regenerate had a significantly ($p<0.05$) greater value compared with groups 1, 4, 5 and amounted to $(26.75\pm0.74)\times10^{-6}$ m. The average thickness value of a stratum corneum layer located on the surface of the epidermis, covering the regenerate, in group 6 was $(3.12\pm0.24)\times10^{-6}$ m, which was significantly lower ($p<0.05$) compared with groups 1, 4, 5. This fact, as in group 2, indicates that the gel with carbon dioxide extract of hops activates the separation processes of horny masses from the skin surface.

The average thickness value of a connective tissue layer in group 6 was $(726.18\pm44.35)\times10^{-6}$ m, which was significantly ($p<0.05$) higher compared to the same indicators in groups 4 and 5.

A comparative analysis of the obtained data has showed that the gel with carbon dioxide extract of hops, firstly, intensifies separation processes of the horny masses from the epidermis surface; secondly, stimulates the proliferative activity of the epithelial layer of the skin, located in the marginal sections of the wound or covers the surface of the regenerate; thirdly, it activates the growth processes of granulation tissue and its transformation into connective tissue, leading to complete healing of the simulated wound of the skin with underlying soft tissues on the 30th day of the experiment.

Observational microscopy in group 7 revealed a wound defect, the depth of which reached the dermis or hypodermis. The wound defect was characterized by a leukocyte-necrotic layer, represented by necrotic fragments of the epidermis and dermis with a large number of dead leukocytes (figure 4). This was followed by a demarcation leukocyte shaft, followed by a layer of granulation tissue with an underlying layer of connective tissue. The maturity of the granulation tissue varied. Thus, we noted predominance of the cellular and vascular components over the fibrous ones in the superficial sections of the granulation tissue adjacent to the demarcation zone and in the deep sections located closer to the layer of connective tissue the fibrous component dominated over the cellular and vascular ones.

In group 7, the average thickness values of the leukocyte-necrotic layer, demarcation zone, granulation tissue layer and connective tissue layer were, respectively, $(228.94\pm25.11)\times10^{-6}$ m, $(149.15\pm11.22)\times10^{-6}$ m, $(319.58\pm19.56)\times10^{-6}$ m, $(78.55\pm11.31)\times10^{-6}$ m.

The epithelial layer in the marginal sections of the wound was characterized by moderate dystrophic changes, in some visual fields it was thinned. Horny masses were found on the surface of the epidermis. The average thickness values of the stratum corneum and epidermis were $(6.87\pm0.92)\times10^{-6}$ m and $(19.54\pm1.11)\times10^{-6}$ m.

In a comparative analysis of the data obtained in group 7 compared with group 4, it was found that the average value of the thickness of the epidermis significantly ($p<0.05$) decreased, the thickness of the stratum corneum layer did not change significantly ($p>0.05$), the average thickness values of the granulation tissue layer and connective tissue were significantly ($p<0.05$) smaller. Thus, the presence of infectious agents in the wound cavity inhibits proliferative processes in the epidermis located in the marginal sections of the wound; inhibits the cleansing of the wound from necrotic tissue; slows down the growth, maturation of granulation tissue and its transformation into connective tissue.

There are also numerous studies in the literature, confirming the presence of infectious agents in a wound as one of the factors, inhibiting reparative processes, leading to the emergence of long-term non-healing wounds that require long-term and complex treatment [15].

In group 8, the survey microscopy and morphometric study did not reveal any differences from group 7, which once again proved that placebo gel did not have a wound healing effect. In this group, the average thickness values of the stratum corneum, epidermis, leukocyte-necrotic layer, demarcation zone, granulation tissue layer and connective tissue layer were $(6.79\pm0.61)\times10^{-6}$ m, $(19.69\pm1.25)\times10^{-6}$ m, $(231.88\pm21.20)\times10^{-6}$ m, $(151.22\pm10.15)\times10^{-6}$ m, $(322.64\pm17.71)\times10^{-6}$ m, $(80.61\pm14.15)\times10^{-6}$ m.

Observation microscopy in group 9 revealed a wound cavity, the depth of which reached the dermis and hypodermis. It was filled with granulation tissue followed by a layer of connective tissue. It is interesting that the granulation tissue in the superficial sections was immature whereas in the deeper sections adjacent to the layer of connective tissue – mature.

The epidermis, located in the marginal parts of the wound, proliferated, formed superficial and deep acanthotic ingrowths into the underlying tissues, crawled to the surface of the granulation tissue. However, complete epithelialization of the wound was not detected. A few horny masses were found on the surface of the epidermis.

In this group, the average thickness values of the stratum corneum, epidermis, granulation tissue layer and connective tissue layer were, respectively, $(3.42\pm0.11)\times10^{-6}$ m, $(26.18\pm1.42)\times10^{-6}$ m, $(452.29\pm21.95)\times10^{-6}$ m, $(101.19\pm17.74)\times10^{-6}$ m. Compared with groups 7, 8 in this group, the average thickness value of the layer of horny masses was significantly ($p<0.05$) smaller and the mean values of the epidermis thickness, the layer of granulation tissue and the layer of connective tissue had significantly ($p<0.05$) large values. Thus, the gel with carbon dioxide extract of hops not only activates separation processes of horny masses from the surface of the epidermis, stimulates the proliferative activity of the epidermis, activates the growth and maturation of granulation tissue with its subsequent transformation into connective tissue, which was shown in group 7, but also activates the processes of purification wounds from necrotic tissues, has an anti-inflammatory, bactericidal and antimycotic effect, normalizing skin microbiocenosis.

CONCLUSION

The complex morphological study has showed that gel with carbon dioxide extract of hops is a highly effective

drug in treatment of severe and very severe acne vulgaris, characterized by the development of deep and infected wound defects. This experimentally created gel activates separation processes of horny masses from the surface of the epidermis, cleaning the pores of the skin; stimulating the proliferative activity of the epidermis, which is located in the marginal sections of the wound or covers the surface of the regenerate; activating the processes of cleansing the wound from necrotic tissue; activating the growth and maturation of granulation tissue with its subsequent transformation into connective tissue. It has anti-inflammatory, bactericidal and antimycotic effects, normalizing skin microbiocenosis**.**

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ORIGINAL ARTICLE

ANALYSIS OF PRIMARY HEADACHES MANAGEMENT IN POLTAVA REGIONS

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ABSTRACT

The aim: Assess quality of diagnosis and treatment of primary headaches (PH) in Poltava region.

Materials and methods: There were examined 195 patients with PH who were previously consulted by different specialists due to headaches. We analyzed previously established diagnoses, previous consultations and prescribed investigations due to headache, drugs that were prescribed for headache treatment.

Results: The misdiagnoses of PH were made due to considering the headache as secondary (as sign of dyscirculatory encephalopathy, arterial hypertension, autonomic dysfunction, cervical ostheochondrosis). Patients older 40 years were misdiagnosed more often with dyscirculatory encephalopathy, while patients under 40 years were more frequently misdiagnosed with autonomic dysfunctions. Patients sought medical help for headache problem and were repeatedly examined by different specialists (general practitioner, neurologist, cardiologist, ophthalmologist, oyorhinolaryngologist, neurosurgeon). Doctors prescribed a large number of identical uninformative neuroimaging and neurofunctional methods regardless of PH nosologies. Also it had been often prescribed therapy with the use of vascular, metabolic, nootropic drugs without specific pathogenetic effects for PH.

Conclusions: It is necessary to improve the diagnosis and treatment of PH according to international standards by raising awareness among general practitioners, neurologists and other specialists about the basics of PH diagnosis and treatment.

KEY WORDS: primary headaches, misdiagnosis, investigations, treatment

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INTRODUCTION

Epidemiological studies conducted in the general population point to average headache prevalence rates of 46% for 1-year prevalence and of 64% for lifetime prevalence [1]. Numerous epidemiological studies conducted over the past decades in most countries of the world, have confirmed the prevalence (90-95%) of primary headache (PH) over secondary ones [2]. PH lead to loss of patients' productivity, to decreases of life quality, to insufficient social, household and labor adaptation, etc [3, 4]. In post-soviet countries including Ukraine, there are still a problem in providing quality medical care to patients with PH, mostly due to inadequate diagnosis and treatment [5]. Not only general practitioners but also many neurologists till now misunderstand the mechanism of PH, considering PH as a symptom of another disease. On the other hand, very often headache sufferers after ineffective consultations lose confidence in doctors and begin to self-medicate [6, 7]. So, for improvement of PH management it is necessary to provide a thorough analysis of typical errors in PH diagnosis and treatment.

THE AIM

The purpose – to assess quality of diagnosis and treatment of PH in Poltava region.

MATERIALS AND METHODS

The study sample comprised the 195 patients who were consulted in educational, diagnostic and treatment center for patients with PH at department of neurological diseases with neurosurgery and medical genetics of Ukrainian medical stomatological academy. The PH diagnoses were established according to The International classification of headache disorder 3rd edition [8]. We analyzed all cases by unified algorithm that included personal data, previously established diagnoses, previous consultations and prescribed investigations due to headache, drugs that were prescribed for headache treatment.

RESULTS AND DISCUSSION

It had been examined 49 cases of episodic migraine (EM), 15 – of chronic migraine (CM), 93 – of episodic tension-type headache (ETTH), 34 – of chronic tension type headache (CTTH) and 4 – of episodic cluster headache (ECH).

As can be seen from Table 1 among patients with EM, CM and CTTH predominated females whereas patient with ECH were exclusively males. Almost all patients were of working age and majority of patients were within most productive age (in fourth or fifth decades of life). In addition, an important feature is the fact that majority of patients with migraine and TTH had a long disease duration (more than 5 years).

Table 1. Characteristics of the study sample

Patients' characteristics		Headache type				
		EM	CM	ETTH	CTTH	ECH
gender	male	14 (29%)	2 (13%)	45 (48%)	11 (32%)	4 (100%)
	female	35 (71%)	13 (87%)	48 (52%)	23 (68%)	-
structure by age, years	18-30	16 (33%)	1 (7%)	11 (12%)	3 (9%)	-
	31-40	20 (41%)	7 (45%)	25 (27%)	9 (26%)	2 (50%)
	41-50	9 (18%)	4 (27%)	32 (34%)	8 (24%)	2 (50%)
	51-60	4 (8%)	3 (20%)	20 (22%)	12 (35%)	-
	61-70	-	-	5 (5%)	2 (6%)	-
headache duration, years	< 1	6 (12%)	-	7 (7%)	-	-
	1-5	14 (29%)	1 (7%)	38 (41%)	3 (9%)	3 (75%)
	5-10	20 (41%)	6 (40%)	37 (40%)	18 (53%)	1 (25%)
	> 10	9 (18%)	8 (53%)	11 (12%)	13 (38%)	-

Table 2. Previous diagnoses of patients with PH

Previous diagnosis	Headache type				
	EM	CM	ETTH	CTTH	CH
migraine	9 (18%)	-	6 (7%)	1 (3%)	1 (25%)
TTH	-	1 (7%)	4 (4%)	2 (6%)	-
dyscirculatory encephalopathy	12 (24%)	5 (34%)	18 (20%)	7 (21%)	-
arterial hypertension	5 (10%)	2 (13%)	16 (17%)	4 (12%)	-
arachnoiditis	-	2 (13%)	1 (1%)	2 (6%)	-
autonomic dysfunction	14 (29%)	2 (13%)	35 (37%)	13 (39%)	2 (50%)
cervical ostheochondrosis	7 (15%)	3 (20%)	12 (13%)	5 (13%)	-
occipital neuralgia	2 (4%)	-	1 (1%)	-	-
trigeminal neuralgia	-	-	-	-	1 (25%)

Table 3. Previous misdiagnoses in patients of different age groups

Previous false diagnosis	Final diagnosis			
	migraine		TTH	
	age ≤ 40 years	age > 40 years	age ≤ 40 years	age > 40 years
migraine	-	-	2 (5%)	5 (6%)
TTH	1 (3%)	-	-	-
dyscirculatory encephalopathy	4 (11%)	13 (69%)	2 (5%)	23 (31%)
arterial hypertension	6 (16%)	1 (5%)	5 (11%)	15 (19%)
arachnoiditis	2 (6%)	-	1 (2%)	2 (3%)
autonomic dysfunction	15 (42%)	1 (5%)	30 (68%)	18 (23%)
cervical ostheochondrosis	6 (16%)	4 (21%)	3 (7%)	14 (18%)
occipital neuralgia	2 (6%)	-	1 (2%)	-

Table 2 demonstrate that the misdiagnoses of PH were made due to considering the headache as secondary (as sign of another disease). As a rule, inadequate diagnosis of PH is the result of ignorance and (or) non-use of International Classification of Headache Disorders. Doctors often make diagnoses that are not included in the International classification of diseases (for example, vegetative dystonia, discirculatory encephalopathy). Diversity and difference of the symptoms of certain PH nosologies do not determine variety of the false diagnoses (structures of the misdiagnoses in patients with different types of PH is more or less identical). For a deeper understanding of the logic of the misdiagnoses we analyzed structure of previous false diagnoses depending on patients age. For this purpose, we have combined all migraine cases as well as all TTH cases.

Table 4. Previous specialist consultations due to headache

Specialist	Headache type				
	EM	CM	ETTH	CTTH	ECH
general practitioner	8	1	15	5	-
neurologist	41	14	78	29	-
cardiologist	16	4	27	9	-
ophtalmologist	10	4	25	4	2
otorhinolaryngologist	3	1	6	2	2
neurosurgeon	-	-	-	-	1

Table 5. Previous investigations due to headache

Investigations	Headache type				
	EM	CM	ETTH	CTTH	CH
head computed tomography	3 (6%)	-	4 (4%)	4 (12%)	-
head magnetic resonance imaging	15 (31%)	9 (60%)	39 (42%)	20 (59%)	3 (75%)
cervical X-ray	7 (14%)	1 (7%)	11 (12%)	5 (15%)	-
rheoencephalography	22 (45%)	9 (60%)	57 (62%)	26 (76%)	1 (25%)
electroencephalography	4 (8%)	2 (13%)	13 (14%)	7 (21%)	1 (25%)
ultrasound of cerebral vessels	8 (16%)	3 (20%)	15 (16%)	9 (26%)	1 (25%)
electrocardiography	13 (27%)	4 (27%)	29 (32%)	7 (21%)	-
blood analysis	6 (12%)	2 (13%)	10 (11%)	5 (15%)	-
urine analysis	2 (4%)	2 (13%)	4 (4%)	2 (6%)	-
blood biochemistry	5 (10%)	3 (20%)	7 (8%)	5 (15%)	1 (25%)

Table 6. Previous prescriptions of drug groups due to headache

Drug groups	Headache type				
	EM	CM	ETTH	CTTH	ECH
diuretics	2 (4%)	1 (7%)	-	2 (6%)	1 (25%)
antihypertensive	6 (12%)	4 (27%)	18 (19%)	10 (29%)	-
antithrombotics	11 (22%)	5 (33%)	16 (17%)	6 (18%)	-
statins	4 (8%)	3 (20%)	13 (14%)	4 (12%)	-
antidepressants	7 (14%)	3 (20%)	20 (22%)	8 (24%)	-
anxiolytics	8 (16%)	2 (13%)	11 (12%)	7 (21%)	1 (25%)
sedatives	10 (20%)	4 (27%)	9 (10%)	9 (26%)	2 (50%)
nootropic	13 (27%)	6 (40%)	22 (24%)	10 (29%)	1 (25%)
cardiac	15 (31%)	5 (33%)	19 (20%)	15 (44%)	1 (25%)
non-steroid anti-inflammatory	9 (18%)	4 (27%)	10 (11%)	5 (15%)	1 (25%)
combined analgetics	7 (14%)	1 (7%)	5 (5%)	3 (9%)	1 (25%)
triptans	5 (10%)	-	3 (3%)	1 (3%)	1 (25%)
anticonvulsants	1 (2%)	-	1 (1%)	-	1 (25%)

As we can see in Table 3, patients older 40 years were misdiagnosed more often with dyscirculatory encephalopathy, while patients under 40 years were more frequently misdiagnosed with autonomic dysfunctions. Thus, an identical clinical picture was interpreted differently depending on patients' age.

Patients were consulted more than 3 times by different specialists in 16 cases of EM (33%), in 13 cases of ECH (87%), in 56 cases of ETTH (60%), in 22 cases of CTTH (65%) and in 3 cases of ECH (75%).

As can we see from Table 4, patients sought medical help for headache problem and were repeatedly examined by different specialists. Significant number of different consultations were the cause, and also the reason of the incorrect diagnoses in patients with PH.

Table 5 shows it had been prescribed a large number of identical investigations regardless of PH nosologies. According to international standards, the diagnosis of PH is entirely clinical based on the analysis of complaints,

anamnesis data, patient objective examination and does not require additional investigations. Neuroimaging and neurofunctional methods are uninformative for PH, do not reveal any pathology and could not indicate the cause or mechanism of headache. For example, it was shown that in patients with normal neurological status, the informative value of computed tomography and magnetic resonance imaging is less than 2% [9]. Excessive prescription of additional investigations without any indications leads to erroneous conclusion about a causal relationship between the detected nonspecific changes and headache, is basis for overdiagnosis of secondary cephalalgias and for prescription of unreasonable treatment. Moreover, additional examinations are prescribed without specific indications, as if "out of habit".

From the point of evidence-based medicine, among the listed agents, only analgesics and triptans can be used for abortive treatment of headaches. But on the other hand, it was recorded relatively large number of *abuse headache cases due to* chronic overuse of medications for abortive treatment of headache (15 cases due to non-steroid anti-inflammatory drugs usage, 11 cases due to combined analgetics usage, 3 cases due to triptans usage and 4 cases due to simultaneous usage of non-steroid anti-inflammatory drugs and triptans). Important is the fact that in cases of correct migraine or TTH diagnosis, preventive treatment for PH was never prescribed.

The establishment of false diagnoses is the basis for the appointment erroneous therapy with the use of vascular, metabolic, nootropic drugs without specific pathogenetic effects for PH. Moreover, prescribed drugs of various groups could lead to polypharmacotherapy and to various side effects (possibly in the form of a headache).

CONCLUSIONS

1. Among the doctors of Poltava region, as well as throughout Ukraine, management of PH is at an insufficient level.
2. It is necessary to improve the diagnosis and treatment of PH according to international standards by raising awareness among general practitioners, neurologists and other specialists about the basics of PH diagnosis and treatment.

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ORIGINAL ARTICLE

HEALTH STATUS OF INSTRUCTIONAL STAFF OF HIGHER EDUCATION INSTITUTIONS WITH SPECIAL TRAINING CONDITIONS

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ABSTRACT

The aim is to study the level and dynamics of instructor officers' somatic health during the course of employment at Ukrainian higher education institutions with special training conditions

Materials and methods: The study involved male instructor officers of the National Academy of Internal Affairs (Kyiv, Ukraine) of different age groups (n=103), who conduct training sessions with cadets – future police officers. The first age group (up to 25 years of age) comprised 8 instructors, the second one (26-30 years of age) – 11 instructors, the third one (31-35 years of age) – 14 instructors, the fourth one (36-40 years of age) – 16 instructors, the fifth one (41-45 years of age) – 19 instructors, the sixth one(46-50 years of age) – 17 instructors, and the seventh one (over 50 years of age) – 18 instructors. The study was conducted in 2017-2019. The level of instructor officers' health was examined according to the methodology of G. L. Apanasenko, which provides for the calculation of body mass, vital, strength, and Robinson indices, as well as the duration of recovery of heart rate after standard exercise. The research methods: theoretical analysis and generalization of literature sources, pedagogical testing and observation, methods of mathematical statistics.

Results: Insufficient level of health was revealed among instructor officers of all age groups – the level of somatic health of the vast majority of instructional staff (over 75 %) is assessed as "low" and "below average", more than 95 % of instructors are below the "safe zone" according to the methodology of G. L. Apanasenko.

Conclusions: The research shows that the current system of physical training of higher education institutions with special training conditions is ineffective and needs to be refined in order to strengthen the health of instructor officers, increase their efficiency and, in general, improve the effectiveness of training future police officers at higher education institutions.

KEY WORDS: somatic health, instructors, physical training

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INTRODUCTION

In the course of official (teaching) activities, there is a significant number of negative factors on the body systems of instructor officers of higher education institutions, including: low functional mobility, length of working day and week, emotional and mental strain, etc. [1, 2, 3]. These factors lead to a decrease in mental and physical performance, deterioration of a body's major systems (especially cardiorespiratory), and under systematic impact can worsen health and cause various diseases (atherosclerosis, hypertension, coronary heart disease, etc.), which determines the need to exercise in order to prevent their negative effects.

Scientific studies [4, 5] show that over the last decade, the average life expectancy of men has decreased by 2.4 years. The difference in life expectancy of the male population in Ukraine and in Western Europe is 12.8 years, and premature mortality, especially among *working-age* men, is 3-4 times higher than in Europe. More than 70 % of the adult population of Ukraine has low and below average levels of physical health [6, 7]. The structure of morbidity is dominated by chronic non-communicable diseases (cardiovascular diseases, malignant neoplasm, mental and endocrine disorders, allergic manifestations), which are characterized by negative dynamics. The prevalence of cardiovascular pathology has increased 1.9 times over the last decade, oncological pathology – by 18 %, bronchial asthma – by 35.2 %, diabetes mellitus – by 10.1 %. The increase in these diseases is caused primarily by a sedentary lifestyle [8, 9]. At present, the fact of pathology of internal organs as a result of the impact of limited motor activity on the human body is widely recognized – there are virtually no such organs or systems which functions would not change under its influence. Hypodynamic mode of activity causes a person a set of functional disorders that go beyond the locomotor system. They influence the functions of cardiovascular, respiratory, and musculoskeletal systems, as well as change some metabolic processes [10, 11]. Insufficient

Table I. The level and dynamics of indicators that characterize the state of somatic health of instructor officers of different age groups during the course of employment (n=103, X±m)

Age groups	n	Years of the research		
		2017	2018	2019
Body mass index, kg/m^2				
1st	8	23.51±0.51	23.80±0.48	23.96±0.52
2nd	11	23.79±0.49	23.99±0.50	24.12±0.51
3rd	14	24.02±0.47	24.25±0.48	24.53±0.50
4th	16	25.87±0.46	25.96±0.48	26.17±0.49
5th	19	26.21±0.45	26.58±0.49	26.79±0.51
6th	17	26.30±0.48	26.42±0.50	26.84±0.52
7th	18	26.71±0.46	26.85±0.47	26.94±0.49
Vital index, ml/kg				
1st	8	56.19±2.06	55.65±1.88	54.75±1.66
2nd	11	54.79±1.43	54.32±1.47	53.79±1.42
3rd	14	52.81±1.50	51.77±1.53	51.48±1.49
4th	16	53.46±1.34	52.26±1.28	51.17±1.27
5th	19	52.31±1.31	51.45±1.25	51.02±1.26
6th	17	51.89±1.42	51.44±1.40	51.11±1.39
7th	18	51.35±1.39	51.19±1.36	50.89±1.38
Strength index, %				
1st	8	61.94±2.20	60.98±2.36	60.05±1.96
2nd	11	60.38±1.43	58.67±1.39	58.39±1.40
3rd	14	58.26±1.41	58.14±1.42	57.42±1.44
4th	16	57.38±1.45	56.86±1.46	56.25±1.48
5th	19	56.01±1.44	55.67±1.43	55.10±1.43
6th	17	55.31±1.42	55.06±1.40	54.84±1.39
7th	18	54.28±1.38	54.11±1.37	54.02±1.36
Robinson's index, c.u.				
1st	8	80.36±1.95	80.37±1.93	81.20±1.98
2nd	11	81.75±1.37	82.01±1.58	82.80±1.51
3rd	14	83.68±1.45	84.27±1.46	84.57±1.49
4th	16	84.30±1.40	84.72±1.45	85.02±1.52
5th	19	84.81±1.41	85.36±1.40	85.45±1.41
6th	17	85.59±1.44	86.10±1.48	86.37±1.49
7th	18	86.62±1.40	87.23±1.42	87.51±1.43
Heart rate recovery, s				
1st	8	140.43±5.62	139.81±5.69	141.14±5.70
2nd	11	153.44±5.64	156.78±5.73	161.11±5.78
3rd	14	165.56±5.31	171.38±5.29	178.03±5.32
4th	16	167.26±5.40	170.44±5.41	172.25±5.46
5th	19	170.21±5.02	177.79±4.95	180.33±4.93
6th	17	179.37±4.89	181.04±4.86	181.27±4.90
7th	18	181.50±5.13	182.29±5.24	182.84±5.20

physical activity, which is common to educators, leads to a significant reduction in energy consumption, nutrient excess, and, as a consequence, to excess weight [12, 13].

According to the World Health Organization (WHO), the approximate ratios of various factors that ensure and shape human health nowadays include: genetic factors

(heredity) – 20 %, the state of the environment (climate, environmental circumstances) – 20 %, the level of health care (medical care) – 8 %, living conditions and lifestyle (rational work, physical activity, nutrition, personal hygiene, abandonment of bad habits) – 52% [14]. It is the conditions and lifestyle with the use of various elements that relate to all aspects of health – physical, mental and spiritual – which can be considered the key to improving health and efficiency [15, 16]. Physical exercises are of great importance for health promotion and disease prevention [17, 18, 19]. Scientists argue that physical education should provide an excellent level of health, high productivity of law enforcement officers and a range of recreational and rehabilitation measures [20, 21, 22].

However, the analysis of normative documents on the organization of physical training of instructional staff in higher education institutions (HEIs) with special training conditions revealed a number of reasons for reducing the positive impact of physical training on the health of instructor officers, including: insufficient consideration of job factors of scientific and pedagogical staff in directive documents; insufficiently clear order of organization of classes; inefficient use of time spent on physical training; a significant percentage of absences from physical education classes; performance of official duties, orders of commanders in the hours intended for physical training; giving in classes preference to improving the indicators of special physical training and preparation for inspections, etc. The foregoing determines the relevance of the research topic.

THE AIM

The aim is to study the level and dynamics of instructor officers' somatic health during the *course* of *employment* at Ukrainian higher education institutions with special training conditions

MATERIALS AND METHODS

The study involved male instructor officers of the National Academy of Internal Affairs (Kyiv, Ukraine) of different age groups (n=103), who conduct training sessions with cadets – future police officers. The first age group (up to 25 years of age) comprised 8 instructors, the second one (26-30 years of age) – 11 instructors, the third one (31-35 years of age) – 14 instructors, the fourth one (36-40 years of age) – 16 instructors, the fifth one (41-45 years of age) – 19 instructors, the sixth one(46-50 years of age) – 17 instructors, and the seventh one (over 50 years of age) – 18 instructors. The study was conducted in 2017-2019. The level of instructor officers' health was examined according to the methodology of G. L. Apanasenko, which provides for the calculation of body mass, vital, strength, and Robinson indices, as well as the duration of recovery of heart rate after standard exercise. The research methods: theoretical analysis and generalization of literature sources, pedagogical testing and observation, methods of mathematical statistics.

RESULTS

The study of body mass index, which characterizes constitutional peculiarities of instructor officers and is one of the signs of a healthy person, suggests that between 2017 and 2019 there was a tendency to an increase in body weight and, consequently, to deterioration of body mass index among officers of all ages groups. However, no significant difference was found between the average rates of officers within age groups during the study period ($p>0.05$) (Table I). The best values were recorded among instructor officers of the first age group. Comparing the body mass index of officers under 25 and over 50, it can be noted that for instructors of the older age group the value of the index is significantly worse ($p<0.001$). Analysis of the body mass index in comparison with the reference table of ranking values shows that officers from the 1st – 3rd, 6th and 7th age groups have a body mass index at the average level for men, and it corresponds to the age norm. However, a detailed analysis of the data shows that the index values of the officers of these groups are at the lower limit, which allows us to predict that in the near future these officers will be overweight. Instructors of the 4th age group in 2019 and the 5th age group at all stages of the study were "overweight". Studies confirm that the professional activity of instructor officers is characterized by a hypodynamic regime and weight gain during service. This indicates the need to improve the physical training of the teaching staff of HEIs with special training conditions in order to stabilize body weight.

The vital index – the ratio of vital capacity of lungs to body weight – is an important criterion for the reserve of external respiration. A study of the dynamics of this index for instructional staff during the *course* of *employment* gives us the right to argue that the performance of instructor officers of all ages tend to decrease, but did not change significantly during the study ($p>0.05$) (Table I). Comparing the indicators of the studied index in officers of different age groups, it can be noted that a significant difference was found only between the values of instructors under 25 and over 50 years of age ($p<0.05$; $p<0.01$). The highest indicators of the vital index were recorded among instructor officers of the first age group (up to 25 years of age) – at the beginning of the study the value was assessed as "average" (56.19 ml/kg), and at the end – as "below average" (54.75 ml/kg). For officers of other age groups, the vital index was below average. All this indicates a weakening of the functional capabilities of the respiratory systems of the instructional staff, both in the teaching process and with age increment.

Analysis of the strength index (the ratio of the dynamometry of the stronger hand to body weight) found that its values for instructor officers of all ages are reduced in the course of service, but do not differ significantly at the beginning and the end of the study ($p>0.05$) (Table I). Teachers over 50 have the lowest strength index. Analyzing the strength index, it can be noted that only for instructors under 25 its value is below average, and in all other groups, the level of reserves of muscular system functions is assessed as "low".

Table II. The level and dynamics of somatic health of instructor officers during the course of employment at HEIs with special training conditions (n=103, X±m)

Age groups	n	Years of the research		
		2017	2018	2019
1st	8	4.24±0.91	4.09±0.87	3.97±0.89
2nd	11	3.61±0.54	3.52±0.52	3.54±0.57
3rd	14	3.24±0.50	3.11±0.59	2.98±0.64
4th	16	3.17±0.49	3.15±0.50	3.12±0.51
5th	19	3.08±0.43	2.96±0.48	2.94±0.48
6th	17	2.87±0.51	2.76±0.52	2.69±0.54
7th	18	2.45±0.43	2.46±0.48	2.38±0.46

According to the value of the Robinson index (product of resting heart rate and systolic blood pressure divided by 100), we can characterize the criteria for reserve and economization of cardiovascular functions. The decrease of the indicator determines the improvement of a particular system. The study of the value of the Robinson index of instructor officers for the study period found a deterioration in indicators from 2017 to 2019 in all age groups, but there is no significant difference in indicators ($p>0.05$) (Table I). Studies of the dynamics of the Robinson index showed that with increasing teaching experience, the cardiovascular system of instructor officers deteriorates – the value of indicators among officers over 50 is significantly worse than among instructors under 25 ($p<0.001$). Indicators of the functional capabilities of the cardiovascular system of the instructional staff from the 1st – 4th groups are at the average level, and from the 5th – 7th – at a level below average.

A study of the dynamics of the time of heart rate recovery to baseline after 20 squats for 30 seconds shows a decrease in the rate of instructor officers of all ages during the study (Table I). With the increase of the age group of instructor officers there is a deterioration of this indicator, which indicates the weakening of the cardiovascular systems of instructors during the *course* of *employment*. Thus, in 2018 for officers under 25 years of age the average rate of heart rate recovery to baseline was 2 min 21 s comparing to instructors over 50 with the result – 3 min 02 s ($p<0.001$) (Table 1). For instructors of all study groups, the level of functional capabilities of the cardiovascular system in terms of heart rate recovery time is estimated as "below average".

A study of the level of somatic health of instructional staff shows that its value for officers of all ages was decreasing during the study, but no significant difference between the indicators of 2017-2019 was found ($p>0.05$). With increasing age, the level of health of instructors decreases as well ($p>0.05$), but the worst value of the level of physical health was found among instructors over 50 years of age (Table II). It was found that the vast majority of instructors have a level of health below average and low – the overall score according to the method of Professor Apanasenko ranges from 2.38 to 4.24 points.

Analysis of the level of somatic health of the entire surveyed contingent in 2019 showed that 45 officers (43.7 %) had a lower than average level of health, 34 instructors (33.1 %) had a level assessed as "low", 21 instructors (20.4 %) – as "average" and health level of only 3 instructor officers (2.9 %) was higher than average (mostly members of national teams who regularly attended sports clubs) (Fig. 1). It is important to emphasize that during the study period, no instructor was found to have a high level of physical health.

The study suggests that the level of somatic health of instructor officers of HEIs with special training conditions is insufficient to perform the tasks of official activity and needs to be improved.

DISCUSSION

Health is the most important factor in the implementation of an individual life program, which largely determines the implementation of social tasks [23]. According to the World Health Organization, health is defined as a state of a person, which is characterized not merely by the absence of disease or infirmity, but also complete physical, mental and social well-being. Only a person who has a harmonious physical and mental development and is well adapted to the surrounding physical and social environment can be considered healthy [14]. A.V. Maglovanyi et al. [24] present the concept of physical health as a state of the body in which the integral indicators of the basic physiological systems are within the physiological norm and change adequately during an individual's interaction with the environment. Other scientists [25, 26] state that health is the *amount* of reserves of a body, the maximum productivity of vitals while maintaining the quality limits of their functions. Thus, based on the above, it can be stated that a healthy person is able to fully realize their physical and mental abilities and fulfill their social purpose.

Currently, in Ukraine there is a critical situation with the health of the population due to a rapid increase in morbidity, mortality, genetic disorders, rising crime, reduced physical fitness of schoolchildren and students, increasing negative phenomena in politics and international realm, as well as many other factors [27]. In his research papers, G. L. Apanasenko, argues that there is a safe level of physical health (on the border of the third and fourth levels – according to the express method it is 12 points), above which there are almost no endogenous risk factors for chronic somatic diseases, nor the diseases themselves or

mortality caused by them [28]. The scientist notes that over the past 20 years in Ukraine the share of the population in the "safe zone" of health has decreased from 8 to 1 %. For comparison, in the United States up to 80 % of Americans aged 20-59 are in the "safe zone" [6, 28]. The current level of development of society largely actualizes the scientific problems associated with finding ways to preserve and improve public health, including instructional staff of HEIs.

According to many researches [10, 13, 19, 29], physical exercise plays an important role in the prevention of occupational diseases and improving the health of various segments of the population. However, the results of our research indicate a low level of somatic health of instructor officers of HEIs with special training conditions, which is insufficient to perform the tasks of teaching and needs to be improved.

CONCLUSIONS

The research results indicate the negative factors of service activities, as well as inadequate impact of the current system of physical training on the level and dynamics of physical health of instructional staff of HEIs with special training conditions. Most of the surveyed instructor officers (over 75 %) have low and below average physical health, more than 95 % are below the "safe zone". All this requires improving the organization of physical training with research and teaching staff in order to strengthen their health and increase efficiency during the course of employment.

Directions for future research. It is planned to investigate the state of physical health of female instructor officers at HEIs with special training conditions.

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ORIGINAL ARTICLE

STOMACH BEZOARIS, CAUSES OF DEVELOPMENT, DIAGNOSIS AND METHODS OF TREATMENT

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ABSTRACT

The aim: Is to determine the tactics and methods of treatment of bezoars of the gastrointestinal tract.

Materials and methods: From 2001 to 2019, 17 patients were diagnosed with "bezoar".

Results: Due to the "weariness" of the clinic, the diagnosis was made in the first 3 days only for 3 (17,6 %) patients. On the basis of the obtained average pH values for 4 (23,5 %) patients established moderate hypoacid, for 4 (23,5 %) - pronounced hypoacid, for 6 (35,3 %) - anacid. For 5 (29,4 %) patients, the bezoars were withdrawn on the first attempt, while the other 5 (29,4 %) were "lumped". In the course of fibrogastroscopic examination, all patients were diagnosed with impaired motor-evacuation function of the stomach: gastroesophageal and duodenogastric refluxes, presence of passive discharge of the contents of the stomach into the esophagus.

Conclusions: Therefore, the preconditions for the development of bezoars may be: hypo- and anacid, impaired motor-evacuation function of the stomach, chronic gastric ulcer, cognitive impairment. Endoscopic method should be preferred in the treatment, on condition of its failure - laparoscopic gastrotomy with bezoar extraction.

KEY WORDS: gastric bezoar, intragastric pH-metry, endoscopy

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INTRODUCTION

Bezoars are foreign bodies formed as a result of swallowing of substances that are not digested in the stomach and accumulate in the lumen of the gastrointestinal tract (GIT). The most common location for bezoar is the stomach. There are several types of bezoaris. Phytobezoar is the most common type of bezoar, which occurs in about 75 % of all types of bezoar, and is formed as a result of the use of a large number of bezorogenic products of plant origin, including grapes, persimmon (diospirobesoar), dates, figs, dogwood, cherries and sweet cherries, corn, pineapple [1, 2, 3]. Such types are also distinguished: lactobesoir - accumulation of lactose and casein (is common for children) [4]; pharmacobezoir – caused by the use of tableted drugs [5], which include: aluminum hydrochloride gel, intestinal aspirin, sucralfate, cholestyramine, nifedipine [6, 7]; Sebobezoar - is formed by refractory animal fats in the form of fatty conglomerates, shellacobezoar (or pixo- or desmobezoar) - resins and bitumen [1, 8]; trichobezoar - the aggregation of hair between the folds of the stomach [9, 10], which occurs more often in women up to 30 years [11], is associated with mental illness and bad habits: trichotomomania (tearing hair) and trichophagia (swallowing hair), it can "tail" spread to the intestine (Rapunzel syndrome) [12]; polybezoar - mixed genesis [1, 8].

It is believed that risk factors for the development of bezoar are a decrease in gastric motility, which may be due to: gastroparesis, postoperative adhesive disease, cystic fibrosis, intrahepatic cholestasis, diabetes mellitus, hypothyroidism and renal failure. Patients with impaired chewing function, psychiatric illnesses also fall into the risk category of bezoar formation [5].

The publications indicate that this pathology on the early stages of the disease has a low symptomatic clinical picture and depends on the location of the bezoar, its structure and size, duration of the disease. This category of patients is characterized by dyspeptic complaints, weight loss, which appear later. As a rule, bezoars are diagnosed when they cause pylorus obstruction, intestinal obstruction, ulcerative ulcers of the mucous membrane, and subsequently - GI bleeding [13, 14, 15]. Fibrogastroduadenoscopy (FGDS), radiological imaging techniques such as ultrasound (ultrasound) and computed tomography (CT) of abdominal cavity (OCP), direct abdominal radiography, X-ray diffraction are used for the diagnosis of bezoars. The radiographs of the radiographs appear as spotty or homogeneous floating masses [16, 17, 18].

A retrospective multicenter study showed that more than a third of patients were not diagnosed with gastric bezoars during primary endoscopic examination [19], which required additional diagnostic imaging techniques to help establish the diagnosis. Thus, on CT, phytobezoaris appear to be clearly defined, rounded or ovate masses with heterogeneous density [20].

In the treatment of phytobezoars the following methods are offered: chemical dissolution of bezoar (L-cysteine and metoclopramide together with cellulose, papain with cellulose, pineapple juice, saline, soda, hydrochloric acid,

Fig. 1. Phytobezoar stomach

pancreatin, 1-2 % zinc chloride, and even zinc chloride, and cola) [14, 21], removal with endoscopic devices, laparotomy [22, 23], laparoscopic treatment approach is preferred over open surgery [24].

Thus, according to various authors, there is no single concept in the diagnosis and methods of treatment, the criteria for the use of surgical treatment are not fulfilled.

THE AIM

The aim is to determine the tactics and methods of treatment of bezoars of the gastrointestinal tract.

MATERIALS AND METHODS

From 2001 to 2019, 17 patients were diagnosed with "bezoar". The age of patients ranged from 25 to 82 years, the average was 58,0 ± 2,5 years. By gender, there were 13 men (76,5 %), women - 4 (23,5 %). For all patients, this diagnosis was established during treatment in hospitals of different profiles. 12 (70,6 %) patients were over 60 years old with comorbid pathology. In the hospitalization period, 10 (58,8 %) patients had cardiac pathology first, 5 (29,4 %) had neurological pathology, and only 2 (11,8 %) had a gastrointestinal chronic obstruction clinic. were hospitalized in the surgical ward.

For 15 (88,2 %) patients a short-term intragastric pH-metry was used to study the acid-forming and acid-neutralizing functions of the stomach under basal conditions and after stimulation with pentagastrin. The average pH levels in different departments of the stomach were measured using an AG-1rN-M acidogastrograph developed at National Pirogov Memorial Medical University, Vinnytsya (State Registration Certificate No. 6226/2007 dated March 16, 2007).

In most cases, the diagnosis was made endoscopically, for 3 (17,6 %) patients with ultrasound ultrasound, for 2 (11,8 %) - with CT. In 10 (58,8 %) cases, the bezoaras were removed endoscopically, in 7 (41,2 %) operatively, 5 of them (29,4 %) - laparoscopically.

RESULTS

The diagnosis of "bezoar" was established for all patients within 10 inpatient days. In the first 3 days, this pathology was diagnosed only for 3 (17,6 %) patients who were diagnosed with "stenosis of the exit from the stomach of oncological genesis", which was caused by the "weariness" of the clinical picture. In the FGDS study, in one case, sebobezoar was accepted as a malignant tumor, the diagnosis was made during the second study, which was managed to eliminate with the help of laparoscopic gastrostomy.

The patients' main complaints were: unreasonable vomiting, which was usually associated with overeating; early saturation; feeling of stomach overflow after breakfast; burping with "rotten eggs"; weight loss with preserved appetite. There was a diagnosis of "Stenosis of the stomach cancer oncology genesis". In the group of patients with cardiac pathology, complaints came first: breast and epigastric pain, shortness of breath, and dyspeptic complaints - in the second place. For patients with a neurological clinic, the dyspeptic symptoms were hidden by the root syndrome of osteochondrosis of the lumbar spine. Thus, in 15 (88,2 %) cases, this pathology had "hidden" or asymptomatic clinical picture.

In the study of acid-forming and acid-neutralizing functions of the stomach by intragastric pH-metry, moderate hyperacid was diagnosed from 15 (88,2 %) for 1 patient (5,9 %), 4 (23,5 %) - moderate hypoacid, for 4 (23,5 %) - pronounced hypoacid, for 6 (35,3 %) - anacid. During the FGDS in all patients were found signs of impaired motor-evacuation function of the stomach, namely, gastroesophageal and duodenogastric refluxes - for 14 (82,4 %) people, the presence of passive discharge of the contents of the stomach into the esophagus - for 4 (23,5 %).

In the treatment of this pathology, preference was given to endoscopic methods. For 5 (29,4 %) patients, bezoars were withdrawn on the first attempt, the other 5 (29,4 %) - this manipulation was performed several times with the method of "lumping", small residues were moved independently on the digestive tract. For seven (41,2 %) patients, where the bezoars had a dense structure and considerable size, an operative method of treatment was applied, which included gastrostomy and extraction of the bezoar from the lumen of the stomach. 5 (29,4 %) patients had this surgery laparoscopically, 2 (11,8 %) with the clinic of stenosis of the exit from the stomach on the background of cognitive impairment were forced to perform gastrostomy laparotomy access. According to the morphological structure of bezoars, they were divided as follows: phytobezoaris - 9 (52,9 %), where the fruit bones were the basis (Fig. 1); trichobezoaris - 6 (35,3 %), consisting of hair and pieces of matter; sebobezoaris - 2 (11,8 %), the basis of which was the so-called "combined fat" of canned food.

DISCUSSION

During the study it was found that this pathology mainly affects older people. Thus, out of 17 (100,0 %) for 9 (52,9 %) patients, this pathology was more than 75 years old,

characterized by significant comorbidity of the disease, including cognitive impairment, which contributes to the inadequacy of complaints, "hidden" of the clinic and its low symptomatic the course. This statement confirms the morphological composition of bezoaris, in which 9 (52,9%) had phytobezoaris with the basis of the bones of berries and in 6 cases (35,3 %) - trichobezoaris, which were based on hair and pieces of matter.

Our study of acid-forming function of the stomach makes it possible to state that patients with impaired motor-evacuation functions of the gastrointestinal tract, with hypo- and anacid of the stomach, are at risk of developing this pathology.

By far, FGDS has the highest diagnostic value. However, diagnostic alertness should be exercised when conducting other studies, such as ultrasound ultrasound and CT, differential diagnosis with oncopathology, peptic ulcer, etc.

Conservative treatment of these formations is considered unpromising, since the use of various chemical compounds that, according to other authors, dissolve the formation data, can adversely affect the gastric mucosa and other organs and systems of the body.

In the treatment of bezoaris, it is desirable to give preference to endoscopic extraction, which can be performed on the early stages of the disease, when the formation is small, "loose" in structure, amenable to fragmentation ("lumping" method). In the case of involuntary surgery, for the elderly a less invasive method is recommended - laparoscopic gastrotomy with bezoar extraction, which contributes to the early activation of patients and the reduction of postoperative complications in the older age category.

CONCLUSIONS

Therefore, the preconditions for the development of bezoars may be: hypo- and anacid, impaired motor-evacuation function of the stomach, chronic gastric ulcer, cognitive impairment. In the treatment endoscopic method should be preferred, if it is impossible to carry out - laparoscopic gastrotomy with bezoar extraction.

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REVIEW ARTICLE

ABORTION, HUMAN RIGHTS AND MEDICAL ADVANCES IN DIGITAL AGE

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ABSTRACT

The aim: The article analyzes the impact of abortion on human rights and women's health in the light of medical and technological advances of the digital age.

Materials and methods: The methods of research were dialectic approach and general analysis of normative and scientific sources, analysis of the results of studies of women's mental health after abortions, analysis of judicial practice, especially decisions of the European Court of Human Rights, the results of author's own empirical studies, the formal legal method, the comparative legal method and the historical method.

It has been established that there is no strong evidence that abortion negatively affects a woman's mental health, including no evidence that the emotional consequences are deeply personal, or are rather the result of societal pressure. Arguments were refuted about extending the protection of human rights regarding abortion to unborn children and their fathers.

Conclusions: The article emphasizes that the ethical burden on medical workers, especially in jurisdictions that require the approval of a doctor to legally terminate a pregnancy, increases significantly due to information flows and community expectations dictated by new medical advances.

KEY WORDS: abortion, digital age, medical advances, human rights, reproductive health

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INTRODUCTION

Medical ethics have traditionally received much attention, including aspects such as ethical research and presentation of results [1], clinical research ethics [2], balance of patient interests and research consent [3], clash doctors with ethically sensitive situations [4], the moral choice of doctors in sensitive matters and their right to participate or not to participate in certain medical practices, however, in the digital age, all these issues become especially acute. The issues are becoming yet more significant because of both, increasing flows of disparate information that affect all people in society, regardless of profession, and by new advances at the intersection of medicine and technology.

One of the ethically sensitive issues is abortion, which is also closely related to the discussion on human rights. First of all, it related with the right to life, which, of course, is fundamental, but at the same time is not an absolute right and may be limited in some cases. It must also be balanced with other rights, especially when it comes to abortion, with the right to privacy, freedom of choice, human dignity and bodily autonomy of women. An important and controversial aspect is the moment of the beginning of life, since its legal and medical definitions may not coincide. The terms "viable fetus", "premature baby", "newborn", "healthy baby" are used in different contexts and do not always have legal consequences. Moreover, for the implementation and protection of human rights it is extremely important to legally determine who we consider to be the owner of rights, to whom we give legal personality.

A separate issue is the gender aspect of abortion. Reproductive health is not a gender neutral issue. And the main burden, as well as social stigma, is imposed on women. In addition, attempts to give the human rights to the fetus in the womb, and attempts to increase the rights of fathers, are often associated with restrictions on the rights of women.

THE AIM

This article focuses on analyzing the impact of abortion on human rights and women's health in the context of the medical and technological advances in the digital age.

MATERIALS AND METHODS

The methods of research were dialectic approach and general analysis of normative and scientific sources – to form a complete picture of the relationship between sensitive issues, medical ethics, women's health and legal regulation in the digital age, analysis of the results of studies of women's mental health after abortions – to verify the assumptions about personal emotional consequences and public perception, analysis of judicial practice, especially decisions of the European Court of Human Rights – to bring together arguments about protecting and limiting of human rights,

including women's rights and third party rights in terms of abortion, as well as the results of our own empirical studies – to test the perception of ethically sensitive situations by lawyers and doctors, both practitioners and trainees. We also use a formal legal method – for studying legal documents, international treaties, human rights legislation of the European Union and the United States of America; a comparative legal method – to compare abortion laws of different countries; a historical method – for tracking changes in societal attitudes towards abortion and its legal regulation.

REVIEW AND DISCUSSION

For many years, it was believed that an abortion done even during the early stages of pregnancy extremely negatively affects the health of women, both physically and mentally. At the same time, real female mortality rate from complications after non-medical abortions and lack of access to legal termination of pregnancy remains quite high, especially in countries where abortions are socially or legally condemned. In particular, unsafe abortion is the leading cause of death among young women aged 10–24 in sub-Saharan Africa [5].

Modern medicine in many cases makes it possible to get by with minimal intervention and minimal consequences for a woman's physical health, therefore the focus of studying negative abortion consequences has shifted to the woman's mental health. However, there is no strong evidence that abortion actually negatively affects women's mental health. Studies from a decade ago showed that there is a low or moderate risk of adverse mental health outcomes, such as psychological disorders and post-stress conditions. In particular, women who had undergone an abortion experienced an 81% increased risk of mental health problems, and nearly 10% of the incidence of mental health problems was shown to be attributable to abortion; women who had an abortion were more likely to report adverse mental health outcomes compared with women who completed a pregnancy (OR 1.81, 95% CI 1.57 to 2.09) [6], although the study noted that the likelihood of mental complications after an abortion is affected by the desire for pregnancy, combined with social factors that make it impossible to continue, as well as conservative views on abortion. And this casts doubt on the fact that the negative effect stems from the fact of abortion itself, and not, for example, from public condemnation of its fact. Another study found that abortion was associated with small to moderate increases in risks of anxiety (AOR 1.28, 95% CI 0.97-1.70; $p<0.08$), alcohol misuse (AOR 2.34, 95% CI 1.05-5.21; $p<0.05$), illicit drug use/misuse (AOR 3.91, 95% CI 1.13-13.55; $p<0.05$), and suicidal behavior (AOR 1.69, 95% CI 1.12-2.54; $p<0.01$) [7], therefore, it was concluded that abortion may be associated with small to moderate increases in risks of some mental health problems.

However, recent studies disprove this. In particular, a 5-year study, the control group of which consisted of women who were refused abortion on the basis of gestational age, showed that rates of depression are not significantly different between women obtaining abortion and those denied abortion; and rates of anxiety are initially higher in women denied abortion care [8]. Limiting access to abortion services does not have a positive effect on women and does not reduce the number of unwanted pregnancies. In particular, some researchers note that ensuring access to abortion services will not increase the likelihood that women will experience subsequent unintended pregnancies [9]. One of the latest sensational studies has shown that there is no evidence of emerging negative emotions or abortion decision regret; both positive and negative emotions declined over the first two years and plateaued thereafter, and decision rightness remained high and steady (predicted percent: 97.5% at baseline, 99.0% at five years); at five years postabortion, relief remained the most commonly felt emotion among all women [10]. These results were evaluated as evidence that emotions about abortion are related to the personal and, especially, social context, rather than stemming from the abortion process itself.

Thus, this is probably the attitude of society, and not real psychological problems that become the determining factor in the negative emotional consequences of abortion for women. Moreover, it was found that shortly after women were denied an abortion, they experienced higher stress than women who had an abortion [11]. This casts doubt on the benefits of prohibitions on abortion, even if we exclude the argument about the right to personal choice of women.

There are no studies that would reliably show how to separate a woman's truly personal internal emotions from those caused by social disapproval, so that one could evaluate the real emotional consequences and formulate recommendations regarding abortion, and, furthermore, argue that restricting access to abortion reduces emotional harm. Moreover, in conservative and religious societies, where this disapproval is much higher; the decision to terminate pregnancy is assessed as emotionally difficult by women and as extremely wrong by others. As noted, assert that the rate of abortions and the easy acceptance of abortion by a society is directly proportional with the secularization degree of that society [12].

The spectrum of attitudes towards abortion in societies ranges from acceptance to complete intolerance, and their legal provision ranges from a complete ban to full legalization. In recent years, the movement for protecting the right to life of the embryo (fetus) until birth has been gaining strength. An attempt to extend the protection of personal rights to the fetus, which is actually located inside another person, inside the woman's body, leads to a direct legal prohibition or a significant complication of the termination of pregnancy. This leads to absurd norms that extend the prohibition of abortion to violence. Ultimately, this threatens women's rights and negatively affects the reproductive health system, forcing it to be guided by non-medical considerations.

Significant changes to abortion law in the United States of America (USA) occurred in 2019. Several USA states have adopted a number of rules that limit the possibility of

having an abortion, for instance threatening doctors who perform abortions with huge jail terms. This does not take into account any reasons for the woman's reluctance to continue pregnancy, such as conception as a result of rape or incest. This is a worrying trend towards tightening legislation and government interference in the private sphere. It is doubly alarming if we recall that it is happening in a democratic and technologically developed country. At the same time, over 40 European states permit abortion where "there is a risk to the woman's health", and there is "abortion on demand" during the first trimester of pregnancy in over thirty European states [13, p. 557].

The prohibition or restriction of abortion is often justified by protecting the rights of the unborn child. But the problem is not only at what point the fetus begins to be considered a human, but also that such rights inevitably clash with the rights of a pregnant woman. For example, in the case "Vo v. France" the European Court of Human Rights (ECtHR) affirmed that an unborn child is not considered a person whose rights are directly protected by Article 2 of the European Convention on Human Rights (ECHR) [14], that is, the right to life. The court established that the rights and interests of the mother, including her right to life, health and privacy, will have priority. At the same time, ECtHR did not refute that an unborn child may have a "right to life", leaving this as a sensitive issue, at the discretion of the state.

In the case "A., B. and C. v. Ireland", in which the applicants relied on Article 8 of the ECHR, that is, the right to privacy, challenging the law, which imposed restrictions on the prohibition of abortion in the Republic of Ireland, the ECtHR ruled that the right to privacy does not include the right to abortion, although a woman's right to respect for her private life should prevail over other rights and freedoms, including the rights of an unborn child [15]. In addition, the court emphasized that Ireland violated the ECHR because it did not provide an accessible and effective procedure by which a woman could establish whether she has the right to legal abortion in accordance with applicable law. At the same time, the ECtHR noted that there is no doubt about the severity and sensitivity of moral and ethical problems arising in connection with the issue of abortion, as well as the importance of relevant public interests. Many researchers believe that the court should have expressed itself more specifically regarding important issues of human rights and who is the subject of these rights. In particular, the issues such as the status of the fetus under the right to life, and whether pregnancy has a "public aspect", have been left undecided or ambiguous, while great emphasis has been placed on the "role of the margin of appreciation in enabling states to strike their own balance between the fetus and the pregnant woman" [13, p. 556]. In addition, while the ECtHR attempts to portray the margin as a means of respecting domestic morals and cultural values, "the margin of appreciation is applied as a tool of evasion, yet these judicial politics amount to a disproportionate response to the violation of women's reproductive freedom" [16, p. 261].

The case "Paton v. United Kingdom", in which a man sought an injunction to prevent his wife from having an abortion, showed that the issue of the rights of third parties could concern not only unborn children, but also their fathers. In this case, the husband tried to forbid his wife to have an abortion, referring not only to the rights of the unborn child, but also to the right of the father to make a decision as part of the right to privacy [17]. However, as a result it was established that the right to privacy cannot be interpreted so broadly as to extend to the father's statements about his wife's decision to terminate the pregnancy, forbid his wife to have an abortion or forbid the doctors to participate in it.

Thus, the argument about human rights is refuted by the fact that the subject of such rights in terms of abortion is a woman, but not third parties, including unborn children and their fathers.

In the digital age, many sensitive issues are becoming more complex, including those related to medical ethics.

On the one hand, unlimited opportunities open up, such as using AI to advance the health of people, instant exchange of experience of successful research and patient treatment strategies, processing of health-related data about various social groups, etc. In addition, other opportunities are greatly simplified, such as using information technology to improve the situation with abortion information: for instance, to collect faster and more accurate information, such as in the studies mentioned above, or to contact a public organization that provides safe abortion kits and direct instructions for women around the world.

On the other hand, some of the effects of digitalization seem unpleasantly alarming, as if we were in a world of dystopia. For example, the widely known case of Target, in which the company's analysts determined that the client was pregnant and started sending thematic ads before she shared this news with her family. Or numerous cases in which AI, based on user behavior on social networks, determined their interest in children's products and continued to display ads based on this, even if users hid it – for personal reasons, or because the pregnancy was interrupted, or in the case of the appearance of a stillborn child.

The future of medicine in the digital era is associated with individualization of treatment, including specific molecular treatment methods for a particular patient, integrated medical specialties, extremely fast exchange of information between doctors [18], and, probably, universalized medical databases. Improving human viability through technological advances is likely to increase. It is currently approximately 23–24 weeks in developed countries, although the extremely premature infant (less than 28 weeks gestation) and extremely low birth weight infant (ELBW) (< 1000 grams) remain at high risk for death and disability with 30–50% mortality [19]. And this creates a temptation for opponents of abortion to extend the protection of human rights to any fetus that has reached a certain survival threshold (and to all earlier stages of pregnancy), to make the starting point of protection the heartbeat, the degree of fetal formation or the degree of survival of premature babies outside the womb.

At the same time, the legal or actual prohibition of abortion, as well as a significant complication of women's access to safe termination of pregnancy, contribute to inequality and widen the gap, primarily economic, between vulnerable groups. In the digital age, this is further exacerbated by the digital divide. What could turn out to be a blessing for women becomes an additional burden for them. For example, in a number of countries, Internet access in households is predominantly in the hands of men. Therefore, women are deprived of important information about their health that could help them. And even more sadly, such information in the digital age is literally in one click.

The unresolved issue is the balance of human rights, first of all the balance between the protection of the fetus and the respect for a pregnant woman's rights. This is an issue of a wide margin of appreciation of states, despite "the emergence of a European consensus that the balance should fall in favor of the woman, at least when her health or well-being is at stake, or at the early stages of the pregnancy" [13, p. 565]. Although there is no doubt that legally women's rights are key, conservative and religious societies, as well as societies that are polarized regarding ethically sensitive issues, will have a negative effect on personal choice and its consequences.

In countries where a doctor's opinion is required to make a decision to legally terminate a pregnancy, the ethical burden on healthcare providers remains quite serious. In particular, in the United Kingdom, where abortion should be available due to maternal health, doctors have a responsibility to ensure its legality and acceptability. Moreover, ethical evaluative categories are used because two doctors judge in "good faith", that the pregnancy "has not exceeded twenty-four weeks and that the continuance of the pregnancy would involve risk, greater than if the pregnancy were terminated" [20, p. 3]. This ethical burden is increasing in the digital age, given the general emotional fatigue from the daily flows of diverse information.

CONCLUSION

Medical advances in the digital age have both positive and negative effects on sensitive issues, including the issue of abortion. Society's expectations regarding the preservation of life are also increasing, which affects the perception of the right to life, increasing the number of attempts to extend it to unborn children. At the same time, the level of condemnation of abortion remains rather high. Especially in conservative and religious societies, which probably explains the negative psychological consequences for women who terminate a pregnancy.

The issue of balancing human rights is becoming even more acute, given the need to assess the conflict of the right to life and the right to privacy, human dignity and autonomy, which leads to attempts to limit women's rights to protect the rights of third parties. In addition, the problem of the relationship between private life and public interests, including the protection of health, remains unresolved. All this becomes more complicated for emotionally sensitive situations. At the same time, the emotional and ethical burden on medical workers involved in deciding on abortion remains serious in all jurisdictions. In the digital age, this is aggravated by endless information flows and corresponding fatigue.

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REVIEW ARTICLE

DIPHTHERIA: CURRENT PUBLIC HEALTH CHALLENGE IN UKRAINE AND WORLDWIDE (LITERATURE REVIEW)

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ABSTRACT

The aim: Of the paper is to analyze the current views on diagnosis and management of diphtheria in children.

Materials and methods: The data of scientific literature have been analyzed, using the bibliosemantic method of study.

Conclusions: The specific prophylaxis is recommended to prevent the infection. It has cross-protection against different strains.

KEY WORDS: children, diphtheria, prophylaxis, management

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INTRODUCTION

The relevance of the topic is determined by the priority and importance of preserving the health of Ukrainian population by improving the current rate of vaccinated individuals against diphtheria. Diphtheria is assigned to one of the dangerous and life-threatening diseases in humans. Immunization is the only means of creating a favorable epidemic situation [1]. Ensuring of non-susceptibility of people to this infection prevents the incidence and spread of diphtheria in the population.

The WHO reports that epidemic situation in diphtheria morbidity has worsened in the world, particularly in Latin America, where a rise in incidence has not been recorded since 1990s [2]. Diphtheria remains a health threat in India, Venezuela, Yemen, Bangladesh (Cox's Bazar), where the intensive growth of the incidence of this infection began in 2017; 80 % of all reported cases of diphtheria in Latin America were registered in Venezuela. Despite vaccination of Venezuela population, in 2018, 1.2 thousand cases of diphtheria were reported, of which more than 80 cases were lethal. In Haiti, about 250 cases were reported, of which 3 cases were lethal; in Colombia, about 10 cases were reported during the same period, which can be related to forced migration of the population from the countries where diphtheria infection cases are recorded [3-6].

The comparison with the post-Soviet countries, for example, in the Republic of Kazakhstan, showed that long-term dynamics for 1990-2012 is characterized by the outbreaks of diphtheria in the period from 1993 to 1998, when local outbreaks with a peak of incidence in 1995 were recorded (1105 cases, including 31 lethal cases) [7].

According to the WHO reports of 2019, Ukraine is threatened with a diphtheria outbreak due to insufficient stock of anatoxin and low rate of vaccinated population in recent years [8]. In the last 9 years, sporadic cases of diphtheria have been reported in Ukraine annually (with the exception of 2017). In 2010-2018, a total of 56 diphtheria patients, including 12 children and 44 adults were registered. No lethal cases have been reported. The first 2 cases of diphtheria were registered in 2018 in the territory of Lugansk region, controlled by Ukraine. In November, 2019, 23 cases of diphtheria were registered, including 5 cases confirmed by laboratory tests: per 1 case among residents of Lugansk, Khmelnytsky, Ternopil, Zakarpatska regions and Kyiv city. 14 presumptive cases of diphtheria were reported among contact persons in Zakarpatska region and one case in Kyiv. The number of reported cases for the period of 2012-2019 increased 4.6 times: from 5 in 2012 to 23 – in 2019 [9]. In 2019, one case was registered in February (Lugansk region) and in August (Khmelnitsky region). The remaining 18 cases were reported in October. In 2019, among the diphtheria patients 19 cases were reported in adults. By localization of the lesion, in the Luhansk region diphtheria of the eye was reported, and in other cases diphtheria of the throat/ tonsils was registered. According to the results of bacteriological examination, *Corynebacterium ulcerans* was reported in one patient from Kiev, in 18 other cases it was *Corynebacterium diphtheriae*.

According to the Public Health Center, as of the end of 2019, 1542650 adults were vaccinated against diphtheria, accounting for 52.3 % of the planned volume [10].

The last epidemic of diphtheria in Ukraine was reported in 1991-1998. During its time, 495 adults died (MOH data for 1999), but according to the Internet resource, the total number was about 700 people, which is probably a picture in the following 2000- and years and infant mortality. According to the Ministry of Health of Ukraine, in 2003 the incidence among adults in Ukraine decreased by 53 %

and constituted the intensive indicator of 0.33, while the infant diphtheria incidence was 0.44 and exceeded the similar values among the adult population by 25 %. At the end of 2003, the overall mortality rate for diphtheria in Ukraine among adults was 0.8 % and 5.7 % among children. Patients died from severe complications of myocarditis, polyneuropathy, infectious-toxic shock, and all of them were hospitalized late and had their last vaccination more than 10 years ago [11].

THE AIM

The aim of the paper is to analyze the current views on diagnosis and management of diphtheria in children.

MATERIALS AND METHODS

The data of scientific literature have been analyzed, using the bibliosemantic method of study.

REVIEW AND DISCUSSION

Diphtheria is the acute infectious disease caused by toxic strains of corynebacteria and characterized by inflammatory process with the formation of fibrinous film at the site of the invasion of the pathogen, the events of general intoxication due to the entry of exotoxin into the blood, resulted in severe complications, leading to infectious-toxic shock, myocarditis, polyneuritis and nephrosis. It is caused by *Corynebacterium diphtheria*, commonly known as the Klebs-Löffler bacillus [12].

Epidemiology. Human carrier of the toxigenic strain is the source of infection [13]. Diphtheria is spread via contact with airborne respiratory droplets, rarely by contaminated objects (houseware, food). Susceptibility is with contagious index of 10-15%, children aged 3-7 years old are most affected. Immunity is unstable. Retrospective data on the incidence of diphtheria in the territory of Ukraine, which was part of the former USSR suggests that the highest incidence of diphtheria was in 1955 – 93.0 per 100000 population, and compared to 1974, it decreased by more than 1162 times due to vaccination and became 0.08 per 100000 population [14].

The features of the **pathogenesis** diphtheria are [15]:

1. Entry of infection is mucous membranes of the oropharynx, nose, larynx and less frequently, eyes, genital organs, wounds, burns.
2. Multiplication of *Corynebacterium*, production of exotoxin and the action of antitoxin (recovery or formation of carriage).
3. Intracellular penetration of the toxin to the body.
4. Fibrinous inflammation (necrosis, stasis, exudation), depending on the form of diphtheria: a) diphtheritic (oropharynx, wound surface); b) croupous (larynx, trachea).
5. Toxinemia (development of toxicosis) and lesions of the adrenal glands, kidneys, cardiovascular system, peripheral nerves.

Classification of diphtheria is shown in Table I.

Complications of diphtheria:

- Early (at the beginning of the second week): nephrotic syndrome, myocarditis, peripheral paralysis of the cranial nerves.
- Late (at the end of the 3-7 weeks): myocarditis, peripheral paralysis of the spinal nerves (polyradiculoneuritis).

Bacterial carriage:

1. Bacterial carriage of reconvalescents.
2. Transitory bacterial carriage (single-time production of *Corynebacterium diphtheriae*).
3. Short-term: up to 2 weeks.
4. Protracted: more than 1 month.
5. Chronic: more than 6 months.

Diagnostic criteria for oropharyngeal diphtheria

Localized: acute onset, unmarked intoxication, low grade fever (38-39 °C), moderate sore throat in swallowing. Moderate cyanotic hyperemia of the tonsils and oropharynx, without clear border, white-yellow, white-gray exudates, do not go beyond the tonsils in the form of isolated spots or coalesced, dense, adherent to surrounding tissues, bleed when removed or scraped, are tending to grow, regional lymph nodes moderately enlarged, not matted together, non-tender.

Extended: acute onset, moderate intoxication, fever of 39 °C and higher, permanent moderate sore throat, cyanotic hyperemia of the mucous membrane of tonsils, oropharynx; swelling of the mucous membranes, exudates extend beyond the tonsils (pillars, posterior wall of the pharynx, uvula), regional lymph nodes are moderately enlarged, somewhat tender palpable.

Toxic: sudden onset, prominent intoxication, fever of 39-40 °C, significantly enlarged lymph nodes, tender, diffuse hyperemia of the mucous membranes and edema of the nasopharynx, tonsils are coalesced, exudates extends beyond the tonsils (mucous membrane of the cheeks, hard palate), nasal tone in the voice, difficulty breathing (wheezing), swelling of the subcutaneous tissue.

Hypertoxic: sudden onset, highly severe intoxication (vomiting, seizures, loss of consciousness), hyperthermia>40 °C predominate over changes in the oropharynx, fulminant development of infectious toxic shock, life expectancy is unfavorable.

Hemorrhagic: along with the signs of toxic stage II-III diphtheria, exudates becomes hemorrhagic (4-5 days), hemorrhages at the sites of injection, bleeding from the mucous membranes and early evidence of myocarditis.

Diagnostic criteria of nasal diphtheria

Gradual onset, intoxication syndrome is minimal or absent, difficulty in nasal breathing, sanioserous discharges, followed by purulent serosanguineous ones, the appearance of excoriations at the entrance of the nose, rhinoscopy reveals erosions, ulcers, sanguineous crusts or dense, adherent white-grey membrane.

Respiratory diphtheria (true croup):

1. Localized diphtheritic croup (laryngeal diphtheria).
2. Extended diphtheritic croup: laryngotracheitis and laryngotracheobronchitis.

Diagnostic criteria of the respiratory diphtheria

Table I. Classification of diphtheria

Mild form	Localized	Patchy oropharyngeal diphtheria, diphtheria of the nose, eye, genitals, ear, skin.
Moderate form	Localized	Membranous oropharyngeal diphtheria, nasopharyngeal diphtheria, localized croup.
	Extended	Oropharyngeal diphtheria, diphtheria of the nose, eye, ear, genitals.
Severe form	Toxic and hypertoxic	Oropharyngeal diphtheria, diphtheria of the nose, eye, genitals, ear, skin. Extended and descending croup.

Stage of croupous cough (following 2-3 days): moderate intoxication, low-grade fever, dry, sometimes barking cough, loud, hoarse voice, gradual increase of clinical symptoms of laryngeal stenosis.

Stenotic stage (following 2 hours to 2-3 days): moderate intoxication, aphonia, silent cough, stenotic breathing (shortness of breath, involvement of auxiliary muscles), hypoxia (cyanosis of the skin and mucous membranes, tachycardia), agitation.

Asphyctic stage: extremely severe condition, anemia, drowsiness, superficial and frequent breathing, reduced retraction of the intercostal spaces, pale gray skin, cyanosis, cold limbs, dilated pupils, no reaction to the surrounding environment, seizures, loss of consciousness, arrhythmia, hypotension, hypothermia, involuntary urination, defecation; death.

Laboratory verification is bacteriological: bacterioscopy of swabs from the oropharynx (on the border of the affected and healthy mucous membrane), nasopharyngeal and pharyngeal swab for culture; serological: AR, PHAT, ELISA.

In terms of differential diagnosis, consider false croup, which is much more common in the pediatric practice [16].

Acute stenosing laryngotracheitis or false croup (ASLT) is a syndrome of a disease characterized by the airway obstruction, also known as croup. ASLT occurs only in childhood, mainly in children under 3 years of age, and then its incidence decreases from 3 to 6 years and from 7 to 14 years. In children under 6 months of age, this condition does not occur. Boys get sick three times more often than girls.

The main causes of ASLT are viruses (20 %), virus in combination with the bacterium (45 %), mycoplasma (15 %), chlamydia (7 %). Among viruses, parainfluenza viruses are responsible for 45 % of cases; other causes include the following viruses: influenza (18 %), adenovirus (13.6 %), respiratory syncytial virus (3 %). In 2005, Human bocavirus (HBoV) was discovered, which causes ASLT in children aged 3 years and is associated with intestinal dysfunction (vomiting, diarrhea). The cause of ASLT is also pediatric infectious diseases: scarlet fever, pertussis and others. In children aged 3 to 7 years, ASLT can be also caused by a newly discovered metapneumovirus, which combines croup syndrome and inspiratory dyspnea in its clinical presentation.

Epithelial tropism is characteristic to all viruses; however, 2 groups of viruses are distinguished on their ability to cause pathological process:

1. Viruses with specific epithelial tropism (parainfluenza, influenza, rhinovirus, respiratory syncytial virus, bocavirus), which cause pathological process with the destruction of epithelial cells and cause gross morphological changes.
2. Viruses, for which epithelial cells are the primary foci of infection on the site of the entry (adenovirus, measles, rubella, herpes simplex virus).

Pathogenesis of croup syndrome involves:

1. Edema of the mucous membrane of the larynx and trachea;
2. Spasm of the larynx and trachea muscles;
3. Hypersecretion of the glands of the airway mucosa.

Pathomorphological alterations are manifested by hyperemia and swelling of the mucous membrane of the larynx and trachea, accumulation of pathological mass and its transformation into the crusts, especially in the hyposecretory form of the disease. During the microscopic examination of the mucosa, dystrophic changes in the epithelium, its desquamation, as well as necrotic-hemorrhagic and fibrinous-necrotic changes are detected, in case of secondary bacterial infection.

Main clinical manifestations: harsh barking cough, inspiratory stridor, hoarseness.

The clinical course of ASLT is staged. Compensated, subcompensated, decompensated and terminal (pre-asphyxia) stages are distinguished. The onset of the disease is sudden, at night, when stridor and dry high-pitched (barking) cough occurs. Overall agitation, restlessness, sleep disorder, loss of appetite occurs; but at the end of the night, the events of laryngeal stenosis disappear and appear again at night, lasting for several days in succession. However, occasionally, the events of laryngeal are growing at the daytime and laryngeal stenosis of I, II, III degree consistently appears. The occurrence of respiratory distress at night can be explained, perhaps, by the fact that due to the horizontal position of the child in the subglottic space, the swelling of the mucous membrane increases and accumulation of pathological mass in the larynx occurs, which contributes to laryngospasm.

Compensated stage is characterized by agitation, cry and sleep disorder. Stridor and inspiratory dyspnea is characteristic; inspiration is prolonged without the pause between inspiration and expiration in case of child's activity. At rest no inspiratory dyspnea occurs, though a marked increase in cardiac activity as a response to inspiratory dyspnea is noted. At this stage, the act of breathing is rearranging,

providing the body with oxygen. Carbon dioxide irritation of the respiratory center is crucial.

Subcompensated stage is characterized by the growing respiratory distress, inspiratory dyspnea is observed at rest, and if the child is agitated, auxiliary muscles are involved in the act of respiration, which is manifested by the suprasternal, intercostal, and subcostal retractions. The events of heart failure are growing. Chest X-ray reveals enhancement of pulmonary pattern, indicating pulmonary circulation disorder.

Decompensated stage is characterized by the prominent respiratory distress. Sternal and *prelum abdominale* muscles are involved in the inspiration and, consequently, epigastric area is significantly retracted. The increased activity of the respiratory muscles contributes to the increased oxygen deficit, resulting in the development of deep acidosis and disorder of redox processes. Suboxidized metabolic products block enzyme systems, lading to worsening of oxygen disposal. Consequently, cyanosis of visible mucous membranes increases and the skin acquires a marble appearance, which is the ominous sign of circulatory collapse. Blood pressure abruptly declines, pulse becomes weak. On auscultation, respiration in the lungs is attenuated and sometimes even not audible that is caused by respiratory distress.

Pre-asphyxia stage is characterized by superficial breathing of Cheyne-Stokes type, pliable areas of the chest and the epigastric area are not retracted, stridor is not audible. Cough is also not audible. The heart tones are muffled, pulse is almost absent, blood pressure is not determined. Cyanosis is changing to sharp paleness, the patient is unconscious, the pupils are dilated, enophthalmia is observed, involuntary urination and defecation. Untimely medical care can lead to death due to impairment of tissue respiration, caused by hypercarbia, intoxication.

Clinical forms of false croup:

1. Edematic form is characterized by growing severity, unproductive dry barking cough and hoarseness. Forced position of the body in children above 2 years of age is characteristic. On auscultation, respiration in the lungs is attenuated;
2. Spasmodic form is characterized by stridor. During sleep, breathing is smooth, calm; loss of voice occurs upon wake up. Minor or no auscultatory changes;
3. Hypersecretory form is characterized by the cough with viscous sputum, the health state is worsening during sleep due to obturation that causes laryngospasm;
4. Mixed form.

Croup severity assessment on clinical manifestations:

1. Suprasternal retraction, signs of Type I respiratory failure, oxygen saturation is 90 %;
2. Perioral cyanosis, respiratory rate is by 25 % higher the age norm, intercostal retraction, Type II respiratory failure, oxygen saturation is 90 %-70 %. The child requires intensive care;
3. Acrocyanosis, sternal retraction, respiratory rate is by 50 % higher the age norm; oxygen saturation is lower than 70%, Type III respiratory failure. The patient should be transferred to the intensive care unit;
4. Asphyxia, total cyanosis, terminal state of health, arrhythmic respiration, jugular venous distention, respiratory rate is by 70 % higher the age norm, oxygen saturation is lower 50 %.

ASLT should be differentiated from **laryngeal diphtheria (true croup)**, which is characterized by a slow onset, hoarseness, fibrinous membranes, growing respiratory distress. The events of toxicosis, cervical lymphadenitis and edema of tissues are observed. From the very beginning, a productive, but not a dry cough appears; it becomes dry after formation of the membranes. In laryngeal diphtheria aphonia is the hallmark. Finally, findings of bacteriological study are crucial.

Staging is characteristic feature of the progress of laryngeal diphtheria: catarrhal or dysphonic (croupy cough), stenotic (compensated, subcompensated and decompensated) and asphyxial. The initial stage lasts for 1-3 days, the beginning is slow, subfebrile temperature, cough is loud, hoarseness, laryngoscopy reveals swelling and hyperemia of the mucous membranes. The younger the child, the faster is the stenosis with aphonia and respiratory distress. Growing toxicosis, cyanosis, hypoxia is apparent. Laryngoscopy reveals grayish membranes along with hyperemia of the larynx and vocal cords. This stage lasts for 2-3 days. The sub-compensated phase is characterized by persistent stenosis, shortness of breath, stridor at rest, respiratory insufficiency. Decompensated stenosis is characterized by a sudden agitation, pulse wave loss on the inspiration is observed. The asphyxial stage lasts for several minutes, the breathing becomes superficial, overall cyanosis, isolated breaths, bradycardia, respiratory arrest is observed.

ASLT and diphtheritic croup should be **differentiated** from epiglottitis (edema and inflammation of the epiglottis), pneumonia, airway foreign bodies, allergic stenosis, laryngospasm in children with rachitis, spasmophilia. In this case, in addition to the anamnesis, dynamics of the disease, clinical and radiological studies, direct laryngoscopy and bronchoscopy are crucial.

The prognosis for ASLT and diphtheritic croup is serious with lethal outcomes in some cases, even if timely comprehensive treatment is provided.

The ASLT therapy is complex and dependent on the stage of the disease and its form. The treatment involves a pediatrician, otorhinolaryngologist, and resuscitation therapist. Children with stenotic laryngotracheitis should be hospitalized regardless of the clinical form and stage of the disease. During the first hours from the onset of the disease, warm drink, warm compresses around the neck, mustard plaster on the front surface of the neck and chest, warm socks, filled with irritants (e.g, dry mustard) is imperative. These activities have a beneficial effect on the course of the disease and may even stop it at the beginning. In addition, cool mist administration is recommended, similar to acute catarrhal [15-16].

In laryngeal stenosis of the first degree, cool mist administration using the state-of-the-art «Nebulizer» ultrasound devices to inhale «Ventolin» (salbutamol with ambroxolum), hormonal therapy: hydrocortisone dosed at 3-5 mg/

Table II. Differential diagnostic criteria for diseases with croup-like symptoms

Symptoms	Parainfluenza	Diphtheria	Chicken pox	Measles
Onset	Acute, less frequently sudden	Gradual, sequential change of periods	Acute	Acute
Hallmarks	Catarrh of upper respiratory tracts, laryngitis	Barking cough, shortness of breath, respiratory failure	Exanthema	Catarrh of upper respiratory tracts, conjunctivitis, rush
Appearance	Unmarked	Unmarked, skin paleness, cyanosis in 3rd degree stenosis	Polymophous skin rush	Swollen; hyperemia of face, conjunctivitis, exanthema from day 3-5
Catarrh manifestation	Prominent, coryza, cough	Absent	Absent	Prominent
Lethargy, adynamia	Mild	Prominent	Absent	Absent
Coryza	Moderate or prominent	Absent	Absent	Prominent
Cough	Dry, harsh	Barking, followed by soundless	Less common	Dry, productive
Voice	Hoarse	Hoarse, followed by aphonia	Without changes	Could be hoarse
Oropharyngeal lesions	Moderate hyperemia	No	No	Moderate hyperemia, enanthema
Lymphadenitis	No	Regional	No	Often multiple

kg body weight or prednisolone dosed at 1-2 mg/kg body weight for 2-4 days, which can be discontinued without reducing the dose, is advisable. Various decongestant mixtures in the form of aerosols, for example: 0.1 % solution of 1ml adrenaline hydrochloride; 1 % solution of 1ml dimedrol; 1 mg chymotrypsin in 1 ml; 1ml hydrocortisone (25 mg). 2 ml of the above mixture is administered for one inhalation 3 times a day. Other anti-inflammatory mixtures can be used. Antihistamines, multi-purpose drugs, sedative and vitamin therapy is recommended.

Laryngeal stenosis of the second degree requires the increase in the dosage of hydrocortisone from 5 to 10 mg/kg body weight, prednisolone up to 5 mg/kg for 5-7 days. The cool air in the ward is imperative for better activity of the ciliated epithelium. Currently, air humidifiers are widely used. Dehydration and detox therapy at a dose of 20 ml/kg, acritical mixtures to reduce the anxiety of the patient is advocated. Treatment should begin already on admission not to waste time. Prompt administration of spasmolytics to arrest swelling spread (2 %No-Spa® 1-2 mg/kg, papaverin 2 % 0.1-0.2 ml/year of life) is imperative. In croup growing 30 mg/kg mucolvane in 0.9 % sodium chlorideis administered jet intravenously. The use of diazolin is contraindicated, since it increases hyperproduction of the mucous membrane.

Laryngeal stenosis of the third degree requires even more intensive anti-inflammatory, dehydration, infusion and oxygen therapy. The dose of hormonal drugs should be increased, for example, hydrocortisone from 10 to 25 mg/kg, prednisolone up to 10 mg/kg; 2 % solution of euphyllin at a dose of 2-3 mg/kg. To reduce metabolic acidosis, 4% solution of sodium bicarbonate is administered at a dose of 4-5 mg/kg body weight intravenously. Symptomatic therapy is indicated. In the presence of hyperthermia, antipyretic drugs and cooling the child by applying cold to the projection of major vessels is recommended. In most cases, such intensive therapy gives a positive outcome within 2-4 hours. For agitated children intramuscular 0,5 ml aminazine or 1 ml droperidol are recommended for children from 6 months to 1 year of age; from 1 to 4 years of age intramuscular 1.0 ml aminazine or 2 ml droperidol; ½ tab of glycine sublingually 3 times a day can be used for older children. It should be remembered about biological role of calcium, which is the basis of bone tissue, stimulator of nerve impulses, universal regulator of muscle contraction, an important component of coagulation system. Hypocalcemic state is noted in the genesis of laryngospasm in the viral ASLT. Reduce in calcium concentration in blood plasma is associated with the severity of the condition in spasmodic forms. Therefore, it is rational to use medicines containing calcium (e.g., Calcium-D3 Nycomed) at a dose of 1250 mg during 1-2 intakes. The common treatment approach includes broad-spectrum antibiotics on indications. In case of viral etiology of the disease, oseltamivir is recommended in influenza, Novirin in ARVI (from 2 year of age), 50 mg/kg inosine pranobex up to 4 times a day from the first months of life, if necessary. In worsening of the overall health sate, tracheobronchial toilet is conducted by direct laryngoscopy, introducing proteolytic enzymes, hormonal medications, low concentration of antibiotics into the trachea with their subsequent suction together with pathological mass of the trachea and bronchi. In the dry form of ASLT with obstructive crusts, this gives very positive effects. In case of ineffectiveness of the above intensive therapy, intubation is performed using general anesthesia for 3-4 days in children under the age of 3 years, for 5-8 days in children of school age.

If the signs of edema are the forefront of clinical symptomatology, then the emphasis is placed on hormone therapy in age-related dosages as indicated above; in spasmodic form sedatives are beneficial; in hypersecretory form mucolytics are recommended. Table II presents differential diagnostic criteria for diseases with croup-like symptoms [11, 15].

If diphtheria was confirmed by the findings of differential diagnosis and laboratory studies then treatment is provided according to the protocol.

Treatment of diphtheria (according to the international protocols):

Basic therapy with antidiphtheritic serum (ADS) according to Bezredko (doses are in 1000 IU) according to international protocols.

In toxic and hypertoxic forms, 1 dose of ADS is administered intravenously drip-feed together with corticosteroids (30-50 mg single dose similar to prednisolone regimen). In combined forms, the amount of ADS is added. For children in the first two years of life, the dose of ADS is half reduced, compared with older children; for children under 8 years 2/3 of the dose is administered.

Intensification. Antibiotics: erythromycin, rulid, penicillin, cephalosporins, lincomycin, (age doses) for 7-10-14 days.

Supportive therapy: desensitizes, vitamins B, C or ascorutin, irrigation of the oropharynx with disinfectant solution.

Syndromic therapy: detoxification, (5 % glucose solution, 0.9 % sodium chloride solution) cumulative dose is 50-100 ml/kg/day; 5-10 mg/kg hydrocortisone or 1.5-2.5 mg/kg prednisolone in toxic forms, protease inhibitors (10-20.000 U contrycal, Gordox®), 150-500 U/kg heparin (in hemorrhagic syndrome).

Patients that are clinically healthy, with negative bacteriological test (twice within 3 days after the completion of antibiotic therapy, at an interval of 2 days) are ***discharged*** from the hospital on day 14-21 in mild and moderate forms and on day 30-60 in severe form. Surveillance by a pediatrician for 6 months is mandatory [17].

Treatment of carriers: vitamin therapy, antibiotics: 30-50 mg/kg/day erythromycin for 7 days. Ultraviolet irradiation of the tonsils, immune modulators is advocated.

Prevention. Non-specific (hospitalization and sanation of patients and carriers, quarantine at the focus of infection for 10 days (examination, swabs) and disinfection) and specific (DTaP vaccine from 2 months of age 3 times with the interval of 30 days (2, 4, 6 months) at a doseage of 0.5 ml i/m; DTaP revaccination at the age of 18 months, following with DT – anatoxin at 6, 16, 26 years old, then every 10 years [18-19].

We have made the analysis of the absolute and relative number of children vaccinated in 2006 and 2009 (vaccination and revaccination) among the population of Poltava region. The results of this comparison were disappointing. The findings of the comparison revealed the twice lower number of the vaccinated children in almost all positions. Apparently, such a significant decrease could not occur very rapidly or suddenly and it can be assumed that since 2007, a steady tendency towards the reduction of active immunization of children in Poltava region has been formed that was confirmed by the comparison of diphtheria vaccination in 2016 (only 28.4 %), which is by 3.2 times less than in 2006 [20].

We report a clinical case of the 8-year-old male patient V., with lethal outcome in 1993 (archival data from the Poltava Infectious Hospital). The boy was hospitalized in the intensive therapy and critical care unit at Poltava Infectious Hospital.

Complaints on hospitalization: sore throat, general weakness. The epidemic history revealed that the child was from a Gypsy family that partially migrated. The boy was not vaccinated. 7 days have passed since the onset of the disease. At the time of admission, the patient was in the moderate state of health and responded adequately to physical examination and questions. The skin was swarthy, slightly reduced in nutrition. Peripheral submandibular lymph nodes were dense, elastic and slightly painful on palpation, with the diameter of 0.7 cm. Mucous membrane of the pharynx was hyperemic, swollen with white patches, difficult to remove. Respiratory rate was 20 per minute, heart rate of 86 beats per minute. Auscultation of lungs reveals normal breath sounds. The borders of the heart were extended 1 cm to the left; heart auscultation revealed indistinct heart tones, tachycardia. The liver protruded 1.5 below the costal margin. Complete blood count revealed elevated ESR, leukocytosis, neutrophilosis; urinalysis revealed moderate erythrocyturia, leukocyturia, proteinuria. Bacteriological study of mucus from the throat revealed a toxigenic strain of *Corynebacterium diphtheriae*. Consultations that involved an ENT specialist, cardiologist, nephrologist was made. Biochemical and instrumental studies that include ECG, Phono CG, abdominal ultrasound, chest X-ray were conducted. Treatment was made according to the protocol, including administration of anti-diphtheria serum, antibiotic therapy, hormone therapy, detoxification therapy, cardiology therapy. On the third day of the patient's stay in the ICU, the health state dramatically worsened. On examination, the skin was pale, no patches in the throat were noted, and no swelling of the surrounding tissues was observed. Body temperature was 36.8 °C, the pulse was weak, 76 beats per minute, heart tones sharply weakened; ECG showed extrasystoles and splitting of the second heart tone. The liver protruded 3 cm below the costal margin. Coffee ground vomitus was observed. Consultation of leading experts was conducted and therapy was corrected. The child's condition continued to deteriorate and a lethal outcome was verified. There were no differences between clinical and postmortem diagnosis. According to the protocol of the postmortem consultation the diagnosis was made: pharyngeal diphtheria, toxic form. Toxic myocarditis. Necrotic colitis of 3-4 degree. Necrotic nephrosis. Bilateral lower lobe pneumonia. Pulmonary edema. Brain edema. Ascites. Hydrothorax.

CONCLUSIONS

Thus, pediatricians, family doctors, infectiologists should carry out prophylaxis among the population regarding the need for timely vaccination against diphtheria, as it is a reliable measure of preventing morbidity and reduction

the risk of complications and toxic forms, leading to lethal outcomes, especially among children [19-20].

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REVIEW ARTICLE

WAYS TO IMPROVE NATURAL FERTILITY

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ABSTRACT

The aim: Based on the study of the results of retrospective analysis, optimize ways to improve natural fertility.

Materials and methods: Data from the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility were used to study this problem.

Conclusions: With a body mass index > 35, the time reguired for conception increases by 2 times. At the same time, when the body mass index <19, the time reguired for conception increases by 4 times. Increased levels of mercury in seafood are associated with infertility. Smoking accelerates the rate of exhaustion of the ovarian follicular apparatus. When trying to become pregnant, it is advisable to avoid drinking more than 2 servings per day. Caffeine intake over 5 cups/day in women correlates with reduced fertility.

KEY WORDS: fertility, reproductive capacity, infertility, cervical mucus

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INTRODUCTION

In their daily practice, doctors often encounter patients' requests for advice on optimizing conditions for the implementation of their reproductive function. However, to date, there has not been developed single evidence-based clinical guidelines that would clearly regulate this issue. Therefore, at the beginning of 2017, the American Society for Reproductive Medicine (American Society for Reproductive Medicine) and the Society for Reproductive Endocrinology and Infertility (Society for Reproductive Endocrinology and Infertility) published their findings on ways to increase the incidence of pregnancy in family couples / individuals in the absence of of them proven reasons for infertility.

THE AIM

Based on retrospective analysis, optimize ways to improve natural fertility.

MATERIALS AND METHODS

Data from the American Society for Reproductive Medicine and the Society for Reproductive Endocrinology and Infertility were used to investigate this problem. The work envisages measures to enure human health, human dignity and moral and ethical standards in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine and relevant laws of Ukraine (opinion of Danylo Halytsky Lviv National Medical University Bioethics Commission).

REVIEW AND DISCUSSION

FERTILITY AND AGE

Fertility is defined as the ability to procreate. With the probability of conception remaining relatively constant from cycle to cycle in some individuals, in general in the population it is usually the highest during the first months of regular sexual life without the use of contraceptives or the entry of sperm to the female genital tract, then gradually decreases. About 80 % of couples manage to conceive a baby in the first 6 months since the beginning of the attempts. The monthly fertility rate is highest in the first 3 months. Fertility is reduced by about half of the women below the age of 40 compared with persons aged 20 years and older.

Fertility varies among different groups of people and decreases with age in both men and women, but the effect of aging is much more pronounced among females. In women, the chances of getting pregnant are significantly reduced after 35 years. Although men also after age 35 begin to deteriorate spermogram parameters, their fertility is not disturbed to about 50 years.

Infertility is defined as the inability of couples in the reproductive age to conceive a child during regular sexual life without the use of contraceptives throughout the year. The appointment of an examination and treatment at an earlier date may be justified by individuals on the basis of their anamnesis and shown to women who are 35 years of age and over after 6 months of unsuccessful attempts to become pregnant in view of the age-related decline in fertility.

FREQUENCY OF SEXUAL ACTS

Over the past decade, a certain amount of literary data has been accumulated concerning the optimal frequency of sexual relations. While sexual restraint over 5 days may adversely affect sperm count, similar intervals of up to 2 days are associated with their normal density. The false idea is that frequent ejaculation reduces malnutrition. E. Levitas et al. (2015) in a retrospective study, analyzing about 10,000 samples of semen, found that the concentration of sperm and their mobility remained normal even with daily ejaculation.[1] The researchers also found that the above-mentioned indicators for men with oligozoospermia may be the highest in daily ejaculation. Non-spontaneous sexual restraint does not mainly affect the morphology of sperm, but if it lasts more than 10 days, then the spermogram parameters begin to deteriorate. Despite the fact that the definition of indicators of spermogram gives valuable quantitative data, with their help can not make an exact conclusion about the fertility of sperm.

Although evidence suggests that daily sexual intercourse has little benefit in terms of successful fertilization, the need to exercise them on certain days or even at certain times of the day can cause severe stress. Stress associated with attempts to conceive can adversely affect the quality of sexual life, reduce sexual desire, lead to a decrease in the frequency of sexual acts. These events can become even more acute when coitus schedules are scheduled based on a predicted ovulation time [2]. Marital couples should be informed that reproductive capacity is increasing with increasing frequency of sexual acts and is the highest if they occur every 1-2 days. It should be noted that the optimal frequency of sexual relations is determined according to their own preferences.

FERTILITY WINDOW

«Fetal window» or «Fertility window» is a period of time that includes five days before and on the day of ovulation, they denote a 6-day interval that ends on the day of ovulation. Theoretically, the viability of both oocytes and sperm is maximal during this time. From the clinical point of view, the maximum fertility interval can be determined by analyzing the duration of the menstrual cycle (MC), the results of ovulation tests and the characteristics of the mucus of the cervical canal.

A sexually transmitted infection with the highest probability may end with fertilization if it occurs within a 3-day interval ending on the day of ovulation. In a study by A.J. Wilcox et al. (1995) with the participation of 221 fertile women, fertility was maximal if sexual intercourse was preceded by ovulation (in 2 days) (Fig. 1).[3] Another study (Dunson D. B. et al., 2010), devoted to family planning, analyzed the results of two cohorts of the subjects surveyed [4]. In one, basal temperature was measured to determine the ovulation time, while in the other, the content of estrogen / progesterone metabolites in the urine was measured. Probability of pregnancy was the highest if sexual intercourse occurred the day before ovulation, and began to decrease on the foreseeable day of its onset.

Among women who described their MC as generally regular, the likelihood of conception as a result of a one-time sexual intercourse increased during a prolonged period [5]. The probability of clinical pregnancy increased from 3.2 % on the 8th day of the cycle to 9.4% on the 12th and decreased to <2 % on the 21st day of MC. It also shows a reduction in the chances of conception when increasing the age of women (Fig. 2). However, the ability to fertilize increases with an increase in the frequency of sexual intercourse during the fertility window [6]. As it is unpredictable to predict the day of ovulation, it is difficult to use any of the available methods for determining it, family pairs should be encouraged to intensify their sexual life shortly after menstruation and up to ovulation (provided that the MC is regular in women). Due to the fact that the duration of the productive window in women may be different, the probability of conception in them also varies [7]. That is why regular sexual activity is the best advice to increase the chance of a pregnancy.

DEFINITION OF OVULATION

The peak of the fertile period can change significantly even in women with regular MCs. Those who monitor their cycle and monitor changes in cervical mucus, libido, pain and mood can accurately predict the time to ovulate only in half of the cases. Although there is no evidence that the definition of ovulation increases the ability to conceive, the time of sexual intercourse is important and, therefore, should be determined by various methods of predicting ovulation.

A study of cervical mucus is a simple and effective way to determine ovulation (Fig. 3). The estimated probability of conception is the highest, if the mucus is delicate and transparent, but the detection of such secretions is not a prerequisite for the onset of pregnancy. The amount of cervical mucus increases with an increase in the concentration of estrogens in the plasma during 5-6 days preceding ovulation, and reaches its peak within 2-3 days after it. D. B. Dunson et al. (2011) in a prospective study, which included an estimate of 2832 MCs, found that changes in cervical mucus are closely correlated with basal temperature, and with this method, it is possible to predict the peak of the fertile period more precisely than during the menstrual calendar.[8].

Ovulation tests, including luteinizing hormone (LH) levels in urine, are widely used to determine the period of maximum probability of conception. Although numerous studies have confirmed the accuracy of methods for determining the peak of LH in urine in the middle of MC, ovulation can occur at any time during the next 2 days, and false-positive results occur in approximately 7 % of cycles. Although the monitoring of urinary LH levels can help reduce the time interval before conception in pairs with infrequent sexual intercourse, in a large study by J. L. Bigelow et al. (2014) found that changes in cervical mucus during MC can predict the days of the highest probability of fertilization with the same or even greater accuracy than

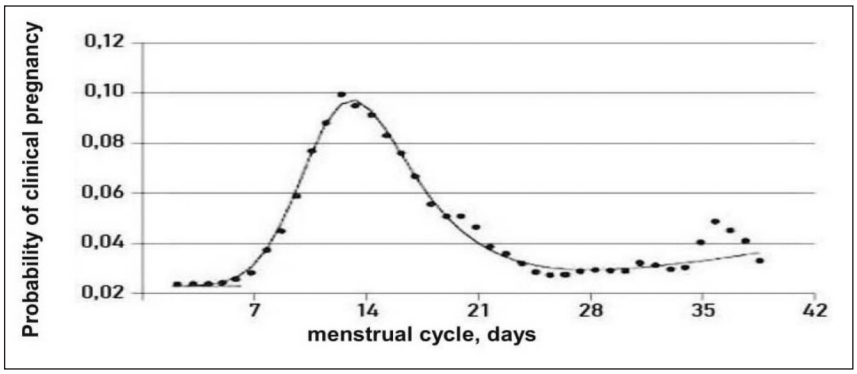

Fig. 1. Probability of the onset of pregnancy depending on the day of the menstrual cycle with a one-time sexual intercourse. Adapted for Dunson et al. (1999).

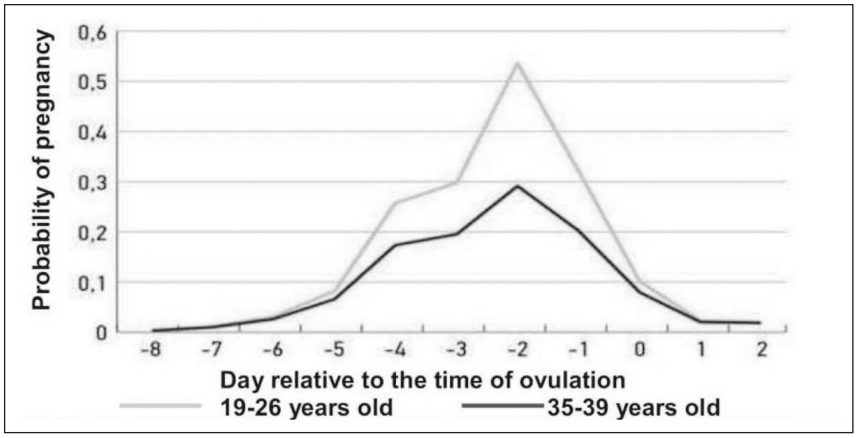

Fig. 2. Probability of the onset of pregnancy depending on the day of the menstrual cycle, including repeated sexual intercourse, in patients of all ages. Adapted for J. B. Stanford, D.B. Dunson (2007).

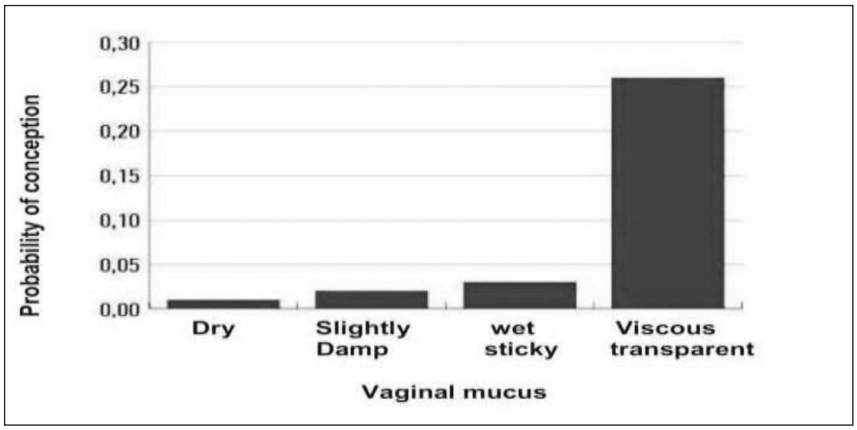

Fig.3. Estimation of the probability of conception on the characteristics of vaginal secretion on the day of sexual intercourse. Adapted for Scarpaet al. (2006).

when measuring basal temperature and monitoring the level of LH in urine.[9].

FEATURES OF THE SEXUAL LIFE OF THE MARRIED COUPLE

Many women believe that staying in a lying position after sexual intercourse facilitates the transport of sperm and prevents sperm out of the vagina. However, this representation does not have any scientific basis.

Spermatozoids can be identified in fallopian tubes after 15 minutes after reaching the cervix in the middle of the MC. After that, they pass through the fallopian tube to the abdominal cavity.

There is not enough evidence to suggest that fertility has an effect on the ability to fertilize during sexual intercourse. It is known that sperm can be detected in the cervical canal a few seconds after ejaculation, regardless of the cohort position. Although female orgasm may contribute to the transport of sperm, correlations between orgasm and fertility are not established. There is also no conclusive evidence of the existence of a link between the characteristics of the sexual behavior of the couple and the child's article.

According to the results of studies on the effects of lubricants on the survival of sperm in vitro, it was concluded that the use of some of them may reduce fertility.While available on sale water-based lubricants inhibit sperm motility in vitro from 60 to 100% for 60 minutes of incubation, canola oil (rape) does not have a similar harmful effect [10]. Unlike mineral oil, a water-based gel-lubricant with no special additives (KY Jelly), olive oil and saliva, diluted to a concentration of <6.25%, negatively affects the mobility and speed of spermatozoa [11]. Lubricants based on hydroxyethyl cellulose also have no noticeable negative

Table I. Life style factors that affect fertility

Factor	Effect on Fertility
Obesity (body mass index> 35)	The time required for conception increases by 2 times
Body mass deficiency (body mass index <19)	The time required for conception increases 4 times
Smoking	The relative risk of infertility increases by 60 %.
Drinking (> 2 servings of drinks / day)*	The relative risk of infertility increases by 60 %.
Caffeine	Ability to fertilize is reduced by 45 %.
Narcotic substances	Relative risk of infertility increases by 70 %.
Toxins, solvents	Relative risk increases by 40 %.

* 1 portion of alcoholic beverage = 10-12 g of alcohol; This is 1 glass of vodka or cognac (25-30 ml) or 1 glass of wine (100-120 ml), or 1 small beer mug (220-260 ml).

effect on spermogram parameters [12]. Despite the above results of studies on the negative effects of some in vitro sperm liquids, their use in couples trying to conceive a child does not interfere with fertilization [13].

Thus, recommended for use in case of need are lubricants based on mineral oil, canola oil or hydroxyethyl cellulose.

DIET AND LIFESTYLE

It is known that fertility among women, both overweight and underweight, is reduced. At the same time, data on the correlation of body weight and fertility in ovulating women is very small (Table 1). Increased levels of mercury in the blood due to the consumption of seafood containing heavy metals is associated with infertility [14,15]. Women planning pregnancy should be advised to take folic acid supplements (a minimum daily dose of 400 micrograms) to reduce the risk of developing neural tube defects [16].

SMOKING

Smoking has been shown to have a negative effect on fertility. In the course of the large meta-analysis (Augood C. et al., 2018), when comparing the health status of 10,928 women smokers with 19,128 non-smokers, it has been found that in the presence of this harmful habit, infertility is much more frequent (odds ratio [VH] 1.60; 95% confidence interval [DII]: 1.34-1.91) [17]. According to research findings menopause occurs on average 1-4 years earlier in female smokers than in non-smokers.[18] This indicates that smoking accelerates the rate of depletion of the follicular apparatus of the ovaries. Although smokers are diagnosed with reduced sperm count, reduced mobility, and morphology, there is no convincing evidence that smoking reduces malnutrition [19,20].

ALCOHOL CONSUMPTION

The effect of alcohol on female fertility is not surely studied. Meanwhile, some scientists came to the conclusion that it has a negative effect, while others believe that its use can increase fertility**.** In a Swedish prospective study involving 7393 women, the risk of infertility was substantially increased (BP 1.59; 95% CI: 1.09-2.31) among those who consumed daily 2 portions of alcoholic beverages.[20] At the same time, this risk decreased (BP 0.64; 95% CI: 0.46-0.90) for those who drank <1 portion of drink per day. Other studies have shown that increased alcohol intake reduces the likelihood of conception [21].

In contrast to the aforementioned data in the Danish study with the participation of 29,844 pregnant women, it was demonstrated that the period before conception was shorter in women who drank wine than those who did not drink alcohol.[22] In a study involving 1769 Italian women in the postpartum period the authors found no link between alcohol intake and conceiving difficulties.[23]

When trying to get pregnant it is advisable to avoid drinking more alcohol (> 2 servings of drinks / day). However, there is limited evidence that its more moderate consumption negatively affects fertility. Undoubtedly, in the course of pregnancy, alcoholic beverages should be completely stopped as they have an established adverse effect on the development of the fetus; At the same time, no «safe» level of their consumption is not defined. In men, the use of alcohol does not aggravate spermogram parameters [24].

USE OF CAFFEINE

Scientists have found that high levels of caffeine intake (500 mg;> 5 cups of coffee or its equivalent per day) correlate with reduced fertility (VH 1.45; 95 % CI: 1.03-2.04) [25]. In general, moderate consumption of caffeine (from 1 to 2 cups of coffee or its equivalent per day) before or during pregnancy does not cause a clear adverse effect on fertility and the effects of pregnancy. In men, caffeine intake does not change spermogram parameters [26].

On the basis of literature review, it was concluded that attending a sauna does not reduce female fertility and is safe during uncomplicated pregnancy [27]. Recommendations for controlling or reducing the testicular heat effects in healthy men are currently not supported [24]

The impact of environmental toxins today is recognized as a potential cause of reduced fertility. Ability to fertilize can be reduced in women who are exposed to certain toxins and solvents, including those used in the dry-cleaning and printing industry. In addition, men who are in contact with heavy metals are more likely to be diagnosed with abnormal

spermogram parameters [28]. Pesticides can negatively affect the reproductive health of agricultural workers [29].

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CONCLUSIONS

- The fruitful window covers a 6-day interval that ends on the day of ovulation, and correlates with the number and characteristics of cervical mucus.
- Frequent sexual intercourse (every 1-2 days) during the «window of fertility» is associated with the highest incidence of pregnancy, but the latter are almost equivalent to less active sexual life (2-3 times a week).
- Accepting certain corytic poses, as well as finding a woman lying in a position after sexual intercourse do not have a significant effect on fertility.
- The use of methods for the detection and prediction of ovulation is appropriate for couples with infrequent sexual relations.
- Moderate alcohol consumption (1-2 servings of drinks per day) and caffeine may adversely affect fertility.
- The period of time required for conception increases with age. For women over 35 years after 6 months of unsuccessful attempts to become pregnant, consultation of the reproductionist is indicated.
- In individuals with regular MC sexual intercourse every 1-2 days from the beginning of the period of the fruitful window can maximize the chances of fertilization.
- Couples who try to conceive a child should avoid smoking, excessive alcohol consumption (> 2 servings of beverages per day), and use of most of the available vaginal lubricants.

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REVIEW ARTICLE

QUALITY ASSURANCE OF MEDICINES: THE STATE AND TRENDS OF THE EUROPEAN UNION AND UKRAINE LEGISLATION DEVELOPMENT

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ABSTRACT

The aim: Scientific substantiation of the state and tendencies of development at the present stage of the legislation of the European Union and Ukrainian legislation on quality assurance of medicines.

Materials and methods: Using the biblio-semantic, systematic-review, analytical, formal-legal and comparative-law methods, the individual regulatory sources of pharmaceutical legislation of the EU and Ukraine were studied.

Conclusions: The paper identifies that pharmaceutical legislation of the EU and Ukraine on quality assurance of medicines at the present stage of their development have common features, which include the number, disorder of existing legal acts, which creates conflicts between them and the potential for gaps in regulation. The main trend in the development of this legislation in the EU and Ukraine is the disparity of legal acts and the lack of significant legislative efforts to codify them.

KEY WORDS: quality of medicines, quality assurance, EU pharmaceutical legislation, Ukrainian pharmaceutical legislation

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INTRODUCTION

The pharmaceutical sector in the healthcare sector of Ukraine is constantly looking for the best ways to integrate into the European and international space. This requires, among other things, continuous efforts to harmonize the pharmaceutical legislation of Ukraine on quality assurance of medicines with the regulatory requirements of international and European legal acts. These processes of harmonization of domestic legislation with international or regional legal sources are not easy for any country, including Ukraine. The reasons for the difficulties are, in particular, the specific features and trends of development of both Ukrainian pharmaceutical legislation and international (regional European) legal sources.

In Ukraine, various aspects of pharmaceutical law have been investigated by pharmacists [1-4], legal scholars [5-8] and representatives of other scientific fields [9] in different years. However, the current peculiarities and tendencies of the development of legislation on quality assurance of medicines at the regional European and national Ukrainian level require additional scientific attention. Scientific interest in these issues is also substantiated by the fact that Ukraine at the level of its Constitution has determined the irreversibility of the European and Euro-Atlantic course, the strategic course of the country for the acquisition of full membership of Ukraine in the European Union and in the Organization of the North Atlantic Treaty [10]. The course set out in the Constitution of Ukraine together with the provisions of the Association Agreement between Ukraine, of the one part, and the European Union, the European Atomic Energy Community and their Member States, of the other part (hereinafter - the Association Agreement) [11] secondly, the task of harmonizing Ukrainian and EU legislation. This creates the legal basis for modern processes of harmonization of the pharmaceutical legislation of Ukraine with the relevant EU regulatory sources, including the quality assurance of medicines.

THE AIM

The aim of the work is to substantiate the current state and trends of development at the current stage of European Union legislation and Ukrainian legislation on quality assurance of medicines in the context of harmonization of European Union and Ukrainian legislation on quality assurance of medicines.

MATERIALS AND METHODS

Using biblio-semantic, systematic-review, analytical, formal-legal and comparative-legal methods, scientific elaboration of separate regulatory sources of pharmaceutical legislation of the EU and Ukraine, which determine the legal basis for quality assurance of medicines at the main stages of their life cycle, was carried out.

REVIEW

The evolution of EU pharmaceutical law is driven by the integration tendencies of creating a common internal market in European countries, including the market for medicines with corresponding positive economic effects for medicines developers and manufacturers, their distributors and end-users. In addition, a significant impact on the development of EU pharmaceutical legislation was made by the ongoing search for the best ways to address the fundamental challenges of increasing the level of social protection, raising the standard of living and quality of life of the countries that initially joined the European Economic Community and later the EU.

Since the 1960s, as a result of regional European and global harmonization of pharmaceutical law, the rules for preclinical study and clinical testing of new medicines, their procedure for registration, ICH and RIS / S recommendations, GLP, GCP, GMP, GDP, GPP, GVP, industry inspection system, etc. In recent years, harmonization has spread to the wholesale and retail of medicines, advertising, quality control organizations. The program of medicinal substance international names choice, methods of efficacy and safety evaluation of medicines, harmonized approaches to quality assurance, principles of marketing promotion have been successfully used [9].

One of the central legal sources of modern EU pharmaceutical legislation is Directive 2001/83 / EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use [12]. However, this source applies along with many other directives, regulations, decisions of EU governing bodies, such as: Directive No 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use; Commission Directive No 2003/94/ EC of 8 October 2003 laying down the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and investigational medicinal products for human use; Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004; Regulation (EC) No 141/2000 of the European Parliament and of the Council of 16 December 1999 on orphan medicinal products; Regulation (EC) No 1901/2006 of the European Parliament and of the Council of 12 December 2006 on medicinal products for paediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/20/EC, Directive 2001/83/EC and Regulation (EC) No 726/2004; Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency etc. [5].

A study of the texts of these and other sources of EU pharmaceutical law has shown that the legal rules governing the quality assurance of medicinal products in the EU are not, at present, merged into one single systematic source, but scattered across different legal acts that form the totality of EU pharmaceutical legislation . For example, the terminological analysis of the text of Directive 2001/83 / EC of 6 November 2001 showed that the expression «quality of medicines» in different variants of terms is used 13 times in different parts of this legal act.

The analysis of not only this European source, but also of other acts, including international acts, indicates a similar approach of the drafters of legal sources to the definition of the legal bases for quality assurance of medicines. Relevant legal provisions are set out in international and regional legal acts, whose call is, first of all, to regulate the legal relations and the legal regime of medicines in general or of certain types of medicines at a certain stage of their life cycle.

This is typical of the GxP guidance system. For example, Good Pharmacy Practice (GPP), which defines the requirements for the retail medicines phase, contains only one mention of the term «medicines quality». However, this provision is crucial because it requires states at national or, where appropriate (for example, at state or provincial level) to establish a legal structure that, among other things, ensures the integrity of the supply chain and the quality of medicines [13].

Thus, at present, a considerable number of legal provisions defining the requirements for quality assurance of medicines at all stages of their life cycle have been accumulated in sources of EU law and pharmaceutical law, which belong to different types of legal sources and have been approved in different years. The main tendency of the legal ordering of the quality of medicines is to preserve the dispersion of the relevant norms by different legal sources and the absence of significant law-making efforts to systematize them in any way in one or more legal acts.

Iven that Ukraine is not yet a member of the EU, Union rules do not apply automatically in Ukraine. Typical decisions in the EU and Ukraine so far, such as the approval of medicines for their introduction into production, their admission to the market and the prevention of risks to patient and public health, are made in different regulatory environments and under different procedures. The competent authority of Ukraine may not participate in a decentralized or mutual recognition procedure for medicines introduced in the EU. To address these and other issues, national legislation should be harmonized with EU law in accordance with the Association Agreement and its annexes [5].

The one of the central terminological constructions in Ukraine that characterizes pharmaceutical relationships at all stages of the medicines life cycle and, accordingly, is reflected in a number of regulatory acts (RAs) is "quality assurance of medicines". On January 23, 2020, we searched the official web portal of the Verkhovna Rada of Ukraine for a search for «quality assurance of medicines», which obtained a list of 694 documents. The request was formed with definitions of the status of the document as «valid»

Table I. Number of approved and valid RAs in Ukraine by time periods

Time period, years	Number of approved RAs that have remained in force as of 23/01/2020	The average annual number of approved RAs that has remained in force as of 23/01/2020
2010 – 2019	478	48
2000 – 2009	182	18
1991 – 1999	29	3
1991 – 2019	**689**	**24**
Before 1991	5	—
Before 1991 – 2019	**694**	—

Table II. Number of approved and valid NPAs in Ukraine by their publishers as of 23/01/2020

№	Document publisher	Number of published documents
1.	Verkhovna Rada of Ukraine (Supreme Council)	140
1.1.	Verkhovna rada of USSR (before 24.08.1991)	*5*
2.	President of Ukraine	12
3.	National Security and Defense Council of Ukraine	2
4.	Cabinet of Ministers of Ukraine	209
5.	Ministry of Health of Ukraine	**157**
6.	Other Ministries	147
7.	Other Authorities	22
	TOTAL:	694

and with search of the given terminological construction both in the title and in the text of the documents.

Important features that were able to be established by analyzing the received list of documents is a significant increase in the attention of the regulatory authorities of Ukraine over the last decade to the legal regulation of pharmaceutical relationships. As the Association Agreement between Ukraine and the EU requires the harmonization of the pharmaceutical legislation of Ukraine and the legislation of the EU, this has become one of the key factors in the intensification of law-making processes by both the Parliament of Ukraine and the regulatory bodies of the executive power.

According to Table I, over the past 10 years (2010 - 2019), 478 RAs have been adopted, and in the previous 19 years (1991 - 2009), 211 RAs have been addressed to the quality of medicines. Currently, only 5 RAs that have been approved until 1991 and contain references to quality assurance have remained in force.

Table II provides information on the number of RAs approved by various Ukrainian authorities. The given number of legal sources includes not only acts developed by state bodies of Ukraine, but also ratified by Ukraine interstate agreements and documents of international organizations. The latter include the United Nations, the World Health Organization, the International Labor Organization, the International Bank for Reconstruction and Development, the European Community and the European Union, the Council of Europe, the European Free Trade Association, etc.

One of the key legal sources for determining the principles of medicines circulation in Ukraine is the Law on Medicines of Ukraine [14]. Along with this it is noteworthy that among the studied RAs there are 122 Laws of Ukraine and 297 orders of the central executive bodies of Ukraine. The aforementioned testifies to the significant dispersion, disorder of the current RAs of Ukraine, which mentions the quality assurance of medicines. They are approved in different years, by different bodies and, of course, are based on different conceptual bases, contain different approaches to the use of terminology, legal constructs, sequence of presentation of legal material, etc. It is quite natural that among such a significant but still not systematic array of legislation there are conflicts of both hierarchical and temporal nature, as well as gaps in the ordering of those legal phenomena and processes that evolve through the practice of ensuring the quality of medicines at different stages. their life cycle.

DISCUSSION

In writing this work, we are aware that along with the terminological constructs "quality of medicines" or "quality assurance of medicines", related and relevant terms are used in the texts of international, European and Ukrainian pharmaceutical legislation, including "safety of medicines", "efficacy of medicines", "suitability of medicines»and the like.

In our opinion, these terms indicate particular aspects of medicines quality and are integral parts of it. The quality of medicines is a generic (integrated) concept that covers the safety of medicines for all interested parties, and the ability of medicines to satisfy the immediate interests of end users, and compliance of medicines with the requirements set out in the legislation and technical documentation [2]. Therefore, the use

of the generic and key constructions «quality of medicines» and «quality assurance of medicines» for the terminological analysis of the legislative arrays of EU and Ukrainian pharmaceutical law is considered quite reasonable.

According to the results of the research, a significant number of disordered, but current RAs always poses a risk to stakeholders who implement their legal requirements in practice. The risk is not only in the inconvenience of using such a large array of RAs created by international and European institutions, as well as by state bodies of Ukraine, but, above all, in the possibility of interested parties violating the contradictory requirements of current legislation. However, such violations are not committed in the form of deliberate actions with predetermined unlawful intentions, but because of an unintentional, erroneous omission of the requirements of a particular RA. As a general rule, such violations provide for more lenient sanctions than for intentional offenses, but the infringing entity is still subject to negative legal liability. As you know, ignorance of the law does not release from responsibility for its violation.

Over the last almost 20 years, Ukrainian scientists have repeatedly put forward initiatives to codify the medical and pharmaceutical legislation of Ukraine [3, 5, 15], but the level of doctrinal projects and proposals has not gone well. Unfortunately, at present there is no work on codification or other systematization of medical or pharmaceutical legislation in the focus of attention of the Verkhovna Rada of Ukraine (the only legislative body of Ukraine [10]).

At the present stage, Ukraine is facing quite difficult tasks that are currently underway to harmonize its own non-consolidated, non-codified array of pharmaceutical legislation with EU law, which is also far enough from being systematically in the classical sense of the concept, due to the regularity of formation and development of continents legal family.

CONCLUSIONS

Thus, pharmaceutical legislation of the EU and Ukraine on quality assurance of medicines at the present stage of their development, despite the considerable number of substantive and formal differences, is characterized by the following common features: the number and disorder of existing RAs of different legal force, approved in different years and by different regulatory authorities, which gives rise to temporal and hierarchical conflicts of data in the RA, as well as gaps in the regulation of certain aspects of pharmaceutical relationships. The main tendency for the development of this legislation in the EU and Ukraine is to maintain the fragmentation of the RAs and the absence of significant legislative efforts to codify them. These legislative features and the tendency to preserve them significantly complicate and delay in time the processes of harmonization of domestic pharmaceutical legislation with EU legislation, create obstacles to the proper legal regulation of the pharmaceutical sector, and also require overcoming, which is the main way we see in the efforts of the systematic or systematic legal framework both in the EU and in Ukraine. This will be the subject of our further research.

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CASE STUDY

COMPLETE THORACIC ESOPHAGUS OBLITERATION: CLINICAL CASE REPORT

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ABSTRACT

Using the example of a clinical case, to present the management features of a patient with complete esophageal obliteration as a chemical burn result, the surgical intervention features in case of a non-standard situation during the operation, and the treatment results analyze.

It was described a clinical case of 41-year-old patient with thoracic esophagus obliteration due to extended post-burn cicatricial esophageal stricture, dysphagia of IV degree in very severe general condition. Stamm-Senn-Kader's gastrostomy was performed as a first step of surgical treatment. Angiography and embolization of the right colic artery and it's branches was performed in 8 months while preserving the middle colic artery. In 20 days the cologastroanastomosis and feeding colostomy on the right chest wall were performed. In 10 days after the colostomy was disattached from the chest wall, the end-to-side esophagocoloanastomosis was performed intrapleurally. In one month after the third surgery and restoration of the food passage by the natural way, closure of the contact gastrostomy was performed. During the observation over the patient (8 years) the postoperative complications were not observed. The patient survived. The proposed staged surgical treatment tactics of patients with complete esophageal obliteration due to post-burn esophageal stricture, dysphagia of IV degree presents effective treatment results and a significant improvement in the patient's life quality.

KEY WORDS: complete obliteration of the thoracic esophagus, esophageal strictures, esophagocoloplasty, colon interponate

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INTRODUCTION

Treatment of patients with extended post-burn cicatricial esophageal strictures is currently considered an unresolved issue that requires further in-depth study to improve treatment outcomes and the patient's life quality [1, 2, 3]. It is primarily connected with the large number of patients with this pathology and complex, long-term treatment, and often it needs to perform difficult reconstructive surgeries, which does not exclude the disability of patients. Very often, patients are employable age people [3, 4, 5]. It emphasizes the social importance of the problem of the diagnostic and treatment management of such patients [1, 2, 3, 6].

For reconstructive surgery of the esophagus, when choosing an interponate, an anatomically and physiologically grounded preference is given to the stomach [1, 6, 7, 8]. «Easing» the complexity of surgery performance for the surgeon and the severity of the operation of esophagogastroplasty is provided by reducing the number of formed anastomoses [1, 6]. For this purpose in case of total esophagectomy, it is possible to perform esophagoplasty by forming a single esophagogastroanastomosis on the neck [1, 6]. According to some authors, esophagogastroplasty is performed in 95% of cases [1, 5, 6, 8].

The stomach can't be used as an interponate in 5% cases of severe extended scarring process in the stomach due to corrosive injuries (chemical burn) or gastric surgeries in anamnesis. In such situations, the colon is used for esophagoplasty [5, 9, 10, 11, 12, 13].

In recent years, esophagoenteroplasty was not performed, as evidenced by the lack of published data and our own experience [1, 2, 3, 6, 14]. This is associated with a higher incidence of postoperative complications and mortality after esophagoenteroplasty, which can be explained by the peculiarities of the vascularization of the small intestine [1, 2, 3, 6, 14].

Using the example of a clinical case, to present the management features of a patient with complete esophageal obliteration as a result of a chemical burn, the features of surgical intervention in case of a non-standard situation during the operation, and the treatment results analyze.

The patient, whose clinical case is described in the presented article, signed an "informed consent" for the processing and the use of personal data.

It was described a clinical case of 41-year-old patient, female, who was admitted to the SI «Zaycev V.T. IGUS NAMSU» with a diagnosis of extended post-burn cicatricial stricture of the thoracic esophagus, dysphagia of IV degree.

CASE REPORT

In the anamnesis 2 months before admission to the hospital, the patient drank aggressive fluid (concentrated

Fig.1. X-ray examination of the upper part of GIT

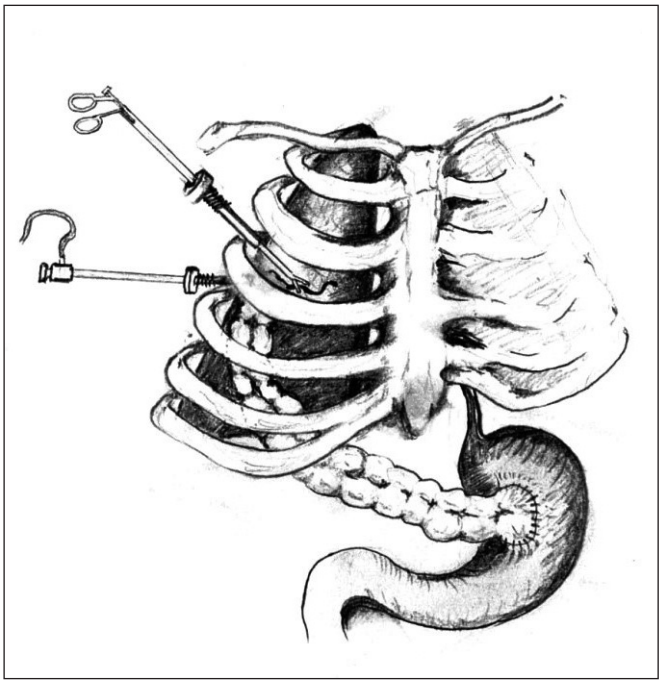

Fig. 2. Thoracoscopic translocation of the proximal part of the mobilized colon interponate

alkaline solution) by mistake, as a result of which she underwent symptomatic infusion therapy at regional hospital. Gradually, the dysphagia progressed, in connection with which the patient was transferred to the SI «Zaycev V.T. IGUS NAMSU». At the time of the first admission to our hospital the patient was in very severe general condition. The patient complained of inability of liquid drinking, weight loss, weakness, dizziness. The patient's weight was 38 kg. The body mass index (BMI) was 16.9 kg/m2 [15, 16]. Nutritional status was very poor. The patient was exhausted. There was not observed any comorbidity in such case.

In the department the patient had got the adequate infusional therapy to restore the homeostatic parameters and parenteral nutrition by intravenous feeding through the central venous catheter.

On X-ray examination of the esophagus it was revealed complete obliteration of the thoracic esophagus, starting from the upper thoracic region (Figure 1).

It should be noted that the radiopaque solution agent evacuation even in 24 hours after the beginning of this diagnostic procedure did not occur.

Due to the severe condition of the patient and her exhaustion, Stamm-Senn-Kader's gastrostomy was performed under the local anesthesia, like a first step of surgical treatment. Intraoperatively the assessment of the stomach and duodenum was performed. Their affection in this case was not observed.

The providing of enteral nutrition was started immediately at the same day of surgery. As an initial enteral nutrition we used 5% Glucose and 0,9% NaCl solutions, and enteral feeding solutions.

There were no complications in the postoperative period after Stamm-Senn-Kader's gastrostomy. The patient was discharged from the hospital on the fifth postoperative day with a significant improvement of her general condition. The patient was instructed how to take care over the gastrostomy and how adequately to feed herself with support of fluid and electrolyte and nutritional balance. After that the dilatational procedures were performed during eight months after gastrostomy formation. But it was ineffectiveness.

The management features for such patient included every months medical check-ups with passing life quality assessment questionnaire (Patent of Ukraine №103176: method of the life quality and treatment effectiveness assessment of patients with gastrointestinal diseases) [17].

In eight months after the gastrostomy formation and relative restoration of the nutritional status, the patient was admitted into SI «Zaycev V.T. IGUS NAMSU». The patient had inability to drink the liquids per os. She provided the enteral feeding and drinking per gatrostoma by herself. Her body weight was restorated to 55,2 kg, the BMI – 24,5 kg/m2 [15, 16]. This parameter control helped to present the changing of BMI Category from "Moderate Thinness" on the first admitting to our Institution to category "Normal" BMI during eight months (according to the World Health Organization's (WHO) body weight recommendations based on BMI values for adults) [15, 16, 18, 19, 20, 21].

After additional examination it was defined that due to the previously formed gastrostomy and followed stomach deformation by the cicatricial process, the stomach could not be considered as an interponate for the affected esopha-

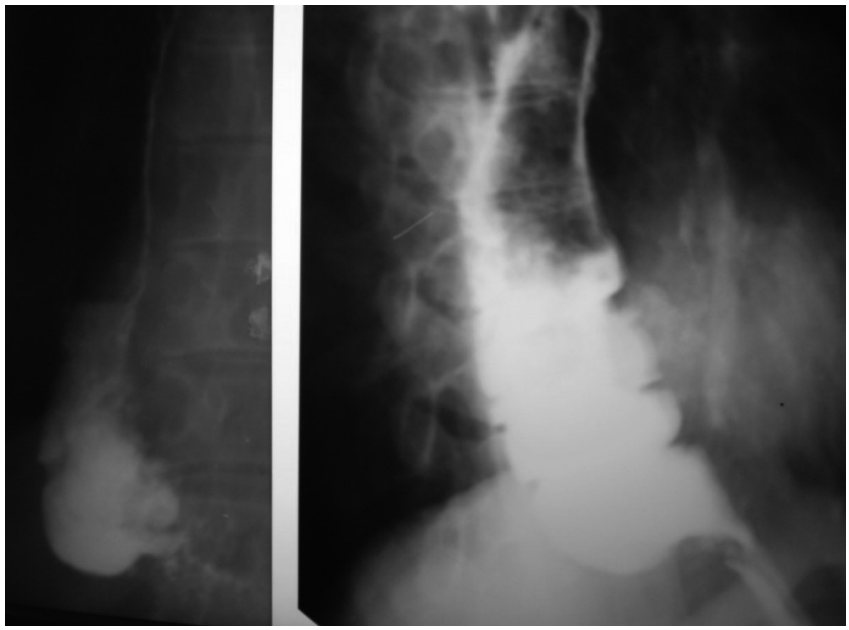

Fig.3. X-ray examination of the esophagocoloplasty segment

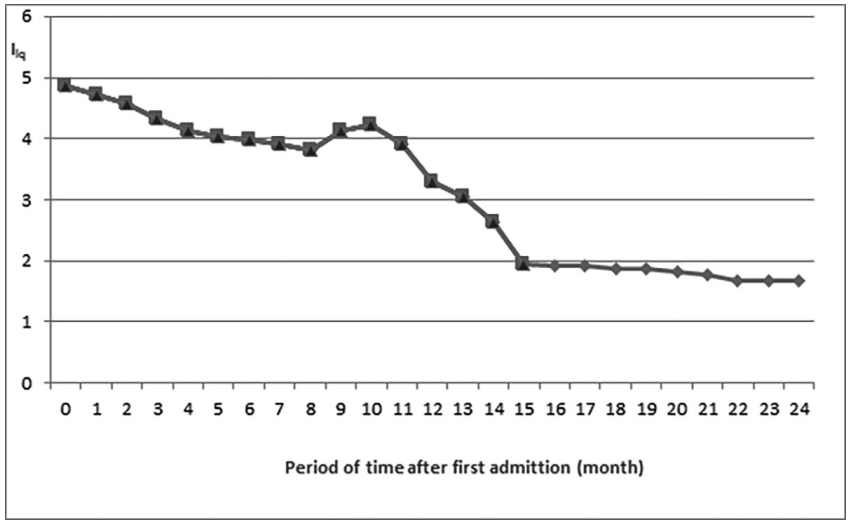

Fig. 4. Dynamics of Iiq (the integral indicator of the patient's life quality)

gus replacement. It was decided to perform esophagoplasty with the right colon on the feeding pedicle from the middle colon vessels with the conducting of the interponate in the isoperistaltic direction intrapleurally.

To clarify the anatomical features of the colon angioarchitectonics before the elective reconstructive surgery, the patient underwent an angiographic study, which aimed to specify the type of the colon blood supply, as well as preventive partial sealing of the supplying colon vessels (by embolization) for the development of the main collateral arterial arcade and adaptation of the colon segment, selected for the interponate mobilization, to the new conditions of blood supply [22]. Angiographic examination was performed according to the classical method of Seldinger through the femoral artery with the introduction of catheters into the superior and inferior mesenteric arteries. The type of structure, condition and course of the superior mesenteric artery, iliac, right, middle and left colic and sigmoid arteries, the presence and state of intervascular anastomoses were evaluated. Occlusion of the right colic artery and it's branches was performed with the help of polyurethane foam balls, Gianturco spirals and crushed pieces of foam while preserving the middle colic artery. No complications due to this intervention were observed.

In 20 days, after the adaptation of the colon interponate to the esophagocoloplasty, the next step of surgical treatment was performed. After laparotomy, the right colon was mobilized with preservation of interponate blood supply by the middle colic artery. Also due to the right colon mobilization, distally side-to-side ileo-transversoanastomosis was formed. After the cologastroanastomosis formation and diaphragmotomy, the proximal part of the mobilized colon interponate was translocated from the abdominal cavity to the right pleural cavity using additional thoracoscopic accesses. Then feeding colostomy was formed from the proximal end of the mobilized colon interponate on the right chest wall (Figure 2).

This technique provides the ability to assess the viability of the proximal end of the colon interponate.

After stabilization of the patient's condition, namely in 10 days after the formation of a colostomy on the right anterolateral chest wall, for the next step of surgical treatment, right anterolateral thoracotomy was performed. During the assessment, the preserved viability of the entire colon interponate was observed. Then, a affected by scarring process, obliterated part of esophagus was mobilized. The azygos vein was ligated. The esophagus was resected within healthy tissues. The colostomy was disattached from the chest wall. The end-to-side esophagocoloanastomosis was performed intrapleurally.

The postoperative period was favorable. The control X-ray examination on the ninth day after reconstructive surgery was performed (Figures 3).

The radiopaque solution passes freely through the interponate. The evacuation of the radiopaque solution through the upper gastrointestinal tract was timely, sings of esophago-colo- and colo-gastro-anastomoses leackages were not observed (Figure 3).

In one month after the third surgery and restoration of the food passage by the natural way, closure of the contact gastrostomy was performed.

We estimated the life quality of this patient by the assessment method of the treatment effectiveness of the patients with gastrointestinal diseases (Patent of Ukraine № 103176) [17]. According to this method, the patient was a subject to this study at different times of treatment: at the first admission to the surgical hospital and then monthly from the moment of the first hospitalization.

The study of assessment the treatment effectiveness of patients and their life quality involves three stages in each case: the patient fills in a questionnaire of subjective data (20 questions) and the calculation of the subjective component of life quality – $I_{sub.c}$.; filling in a questionnaire of objective data by a doctor (10 questions) and calculation of the objective component of quality of life – $I_{ob.c.}$; integral indicator of life quality – I_{lq} (according to the formula: Ilq=(Iob.c.+Isub.c)*0,5) [17].

The patient's examination at different stages of treatment allows us to assess not only the patient's life quality, but also to quantify the treatment effectiveness.

An inverse scale is used to assess life quality, which allows to interpret the results of its quantitative assessment [17]: higher values (1 to 5) obtained on the scale correspond to more pronounced symptoms of the disease, more severe physical and/or psychological condition of the patient and, accordingly, a rather low life quality [17].

Reducing the severity of the patient's condition is reflected in the value decrease of the indicator obtained on the scale. The decrease in the scale reflects the improvement of the patient's condition, reducing symptoms, attenuation of pathological processes. It indicates the improvement of patient's health and respectively, it's life quality [17].

The dynamics of the integral indicator of the patient's life quality (I_{lq}) at different stages of observation depending on the initial admission into the surgical hospital is presented on the graph (Figure 4).

At the time of the patient's first admission to the surgical hospital I_{lq} reflected a «very low life quality» due to the severity of the general physical and psychological conditions of the patient and her exhaustion. After the formation of a contact gastrostomy during the first eight months of the observation, a steady improvement of I_{lq} was observed. At this stage, the patient was admitted for a more thorough examination and preparation for the next surgical steps, the implementation of which during the period from 8 to 10 months explains the temporary deterioration of I_{lq} to «very low life quality» at the time of recovery after complex staged surgical treatment. It should be noted that $I_{sub.c.}$ throughout the study up to 11 months after the beginning of the observation "suffered" due to the presence of stoma and psychological discomfort provoked by it, and with further observation, the indicator $I_{sub.c.}$ markedly was improved after the closure of the stoma. From the 11th to the 14th month there was a constant improvement of I_{lq} to "low quality of life", and from the 15th month (the graph shows the absence of red color) there was a stabilization of the patient's general condition and I_{lq} improvement to "satisfactory life quality" (Figure 4).

The graph reflects, that as the treatment course result, the patient showed corresponding changes in both objective and subjective components and an integral indicator of life quality at each stage during the observation. After all stages of surgical treatment, a stable, even improvement in life quality to a satisfactory level was reflected. The treatment had a positive effect and the life quality gradually was improved. The treatment course can be regarded as effective.

The patient was under the observation for 8 years, no complications were detected, the nutritional status of the patient was completely restored.

Patients with complete esophageal obliteration due to the extended post-burn cicatricial esophageal strictures and, dysphagia grade IV are admitted into surgical department in a state of exhaustion, sometimes cachexia. Radical restoration of the food passage in a natural way at this stage is impossible due to the severity of the general condition, which necessitates two- and sometimes three-step surgical treatment. Theoretically in such situations, surgeons consider a two-step approach to the treatment tactic [23]. At the first stage, gastro- or enterostomy is performed to provide enteral nutrition, restore nutritional status and stabilize the general condition. In our practice, we give the preference to the contact gastrostomy formation in such cases. Gastrostomy provides a more physiological digestion while maintaining the anatomical sequence of the food bolus passage through the gastrointestinal tract, which allows for a more efficient and faster restoration of the patient's nutritional and general status. In the presented clinical case, the patient needed an enteral nutrition. It was decided to perform Stamm-Senn-Kader's gastrostomy, considering the early terms of cicatricial changes and the incompleteness of the pathological process of scarring of the esophagus, as well as the inability to assess the degree of stenosis of the esophageal lumen in the future. Also, in this situation, the option of performing a gastrostomy with the simultaneous formation of a gastric interponate was not considered, since this type of surgery is performed under general anesthesia (laparoscopic or laparotomy) and

the general condition of the patient did not allow this type of operation to be performed.

It should be noted that such patients, after the gastrostomy formation, are subject to dilatational procedures. The effectiveness of dilatational procedures determines the implementation of the next step of surgical tactics. In case of restoration of the esophageal patency and nutritional status, the patient does not need surgical treatment, and in this situation, the second step of surgical treatment is the contact gastrostomy closure (without additional surgery). It leads to achievement of the ultimate goal of patient's treatment – the food passage restoration by the natural way.

But in case of residual dysphagia on the base of ineffective dilatational procedures at the second step, resection of the affected esophagus and esophagoplasty, closure of the stoma are performed.

The irreversible loss of the colon interponate is a rare cause of tremendous consequences for the patient. The main cause for the colon interponate loss is it's ischemic necrosis in the early postoperative period. The colon interponate necrosis has been reported in 4% to 8% of patients after esophagocoloplasty. It is typically diagnosed between second and seventh postoperative days in a not doing well patient without apparent reason [5, 9, 10, 11]. Emergency management includes removement of the necrotic segment and a cervical esophagostomy formation. Less common conditions that may lead to colon interponate loss include it's diffuse ischemic stenosis [4, 5], operative trauma, colon interponate necrosis after incarceration due to colon interponate translocation into the pleural cavity [4, 5], and repeated aggressive liquids (caustic, acidic, etc.) injuries[4, 5, 12].

Since the second step of surgical tactics carries the great risks of development intra- and postoperative complications (sometimes fatal), we divide its implementation in the time interval according to the anatomical and physiological grounds into several stages. This decision about the amount of stages is made individually in each case.

During the second step of surgical treatment, due to the risks of ischemia of the colon interponate, it is decided as additional surgical step to form colostomy on the anterolateral right chest wall for observation over the interponate. This approach allows to prevent the occurrence of fatal, irreversible complications in case of development of critical ischemic changes in the form of interponate necrosis. While the observing the state of the colon interponate for about 2 weeks after this surgery, the presence of convincing data on the viability and sufficient adequate blood supply of the colon interponate allows to make a decision about the next step of reconstructive surgical treatment.

This surgery includes the closure of the colostomy and the formation of an esophagocoloanastomosis. It provides the restoration of the food passage by the natural way. Control X-ray examination in early postoperative period make it possible to track the possible development of esophagocoloanastomotic complications. The reconstructive stage of the surgical treatment in the presented clinical case was performed without the gastrostomy closure. Formed at the first step of the surgical treatment, the contact gatrostomy that was used up to this point as a so-called "insurance" and provided enteral nutrition. During the early postoperative period, the gastrostomy continued to perform its functions. The main goal of the gastrostomy preservation at this stage was to provide enteral nutrition in the early postoperative period, especially in the case of postoperative esophagocoloanastomotic complications, such as anastomositis with dysphagia, partial or complete esophagocoloanastomotic leackage. In the presented clinical case, the complications in postoperative period after the reconstructive stage of surgical treatment were not observed. At this step in one month after esophagocoloanastomosis formation the contact gatrostomy was closed.

During the observation over the patient the postoperative complications after each step of surgical treatment were not observed. The patient survived.

Treatment tactics of patients with complete esophageal obliteration due to the extended post-burn cicatricial esophageal strictures until recently remains complex and controversial and requires an individual approach in each case for a long period of time.

CONCLUSIONS

The proposed staged approach in the surgical treatment tactics of patients with complete esophageal obliteration due to extended post-burn cicatricial stricture of the thoracic esophagus, dysphagia of IV degree presents effective treatment results and a significant improvement in the patient's life quality. This retrospective research showed good postoperative results and need to be continued.

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The research is performed according to the scientific subject of the State Institution «Zaitsev V. T. Institute of General and Urgent Surgery of National Academy of Medical Sciences of Ukraine". It is a fragment of researches "To develop the surgical tactics in patients with extended post-burn cicatricial esophageal strictures using transchiatal esophagoplasty" [State registration № 0114U000084], "To develop transchiatal reconstructive surgeries in patients with leackage of esophageal anastomoses, esophageal injures and perforations" [State registration № 0117U000341] and "To develop individual medical and diagnostic tactics for stenotic diseases of the esophagus, taking into account etiological factors" [State registration № 0119U002468].

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CASE STUDY

A RARE BRAINSTEM INFLAMMATORY SYNDROME, CLIPPERS, MYTH OR FACT. CASE REPORT WITH CRITICAL REVIEW

10.36740/WLek202101131

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ABSTRACT
A very rare inflammatory disease of CNS, CLIPPERS syndrome, was recently described and only a few sporadic cases are reported in the medical literature. Its etiology and pathogenesis are unknown, that together with the polymorphic and sometimes confounding neurological manifestations, and radiological findings represent a real diagnostic and therapeutic challenge for clinicians. Aim: To highlight the importance of clear and specific diagnostic assessment. Here we present the case of a 40-year-old male with a subacute lymphocytic midbrain inflammation accompanied by vasculitis. We discuss the symptoms, imaging and treatment of this lesion.

KEY WORDS: brainstem, pons, encephalitis, steroids

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INTRODUCTION

Chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids (CLIPPERS) is a rare CNS inflammatory disorder affecting prominently the brainstem and particularly the pons and adjacent areas such as the cerebellum [1]. It was shown that two main peculiar properties are essential to characterize the case as CLIPPERS; one of them is the classic MRI findings of post-gadolinium enhancing punctate and curvilinear lesions "peppering" in these areas with appropriate clinical manifestation. The second requirement for a correct diagnosis is a dramatic clinical and radiological response to glucocorticosteroid (GCS) based immunosuppression. So, the diagnosis of CLIPPERS is based on clinical presentation, radiographic findings, response to steroids, and exclusion of other diagnoses with imaging, serum and CSF laboratory testing [2].

Although the underlying pathogenesis of this condition is poorly understood and the neuropathological findings are far from specific, there is reason to believe that this pathology has characteristic signs of an immune-mediated inflammatory disorder with a pronounced vascular and perivascular tropism predominantly located in the pons and the peripontine region [3]. However, the ultra-high-field MRI at 7.0T revealed perivascular inflammation not only in the brainstem and cerebellum but also in brain areas with normal imaging on 3.0T MRI [4]. The immune-based nature of CLIPPERS has recently been confirmed. It was determined, CD45+ lymphocytes with a predominance of CD3+ T cells and CD4+ T cells were accumulated in vessel walls and perivascular area of brainstem and cerebellum of patients with CLIPPERS. CD4+ T cells also infiltrated the brainstem parenchyma. The presence of CD8+ T cells was less pronounced and only a few CD20+ B cells were observed [4]. The omics-based approach showed importance of cell adhesion molecules (VCAM-1, ICAM-1) and interleukins (IFN-γ, TNF-β, IL-1, IL-6, IL-10) upregulation, and IgG deposition in the pathogenesis of CLIPPERS but the central role, certainly, belongs to complement activation [4]. This allows them to be considered potential targets for the treatment of CLIPPERS. The monoclonal antibodies are very promising in this regard and first positive results already published [5]. It's tocilizumab – IL-6 receptor-blocker provides the long-standing relapse-free time period even in the patients with various relapses and nonresponse to multiple agents. Immunosuppressive effects of tocilizumab are thought to be a result of the induction and expansion of B-regulatory cells as well as the differentiation of T cells into effector or regulatory T cells with a significant increase of regulatory T cells [5]. In addition, upregulated VCAM-1, ICAM-1, IL-8, and eotaxin in the CSF may be potential biomarkers in CLIPPERS.

In this case report, we demonstrate that early recognition of this syndrome and a directed diagnostic approach make treatment more effective, patient outcomes earlier, and limit or avoid unwanted complications.

CASE STUDY

A 40-year-old male admitted to the Center of Infectious Disorders of the Nervous System, Kyiv, Ukraine (CIDNS)

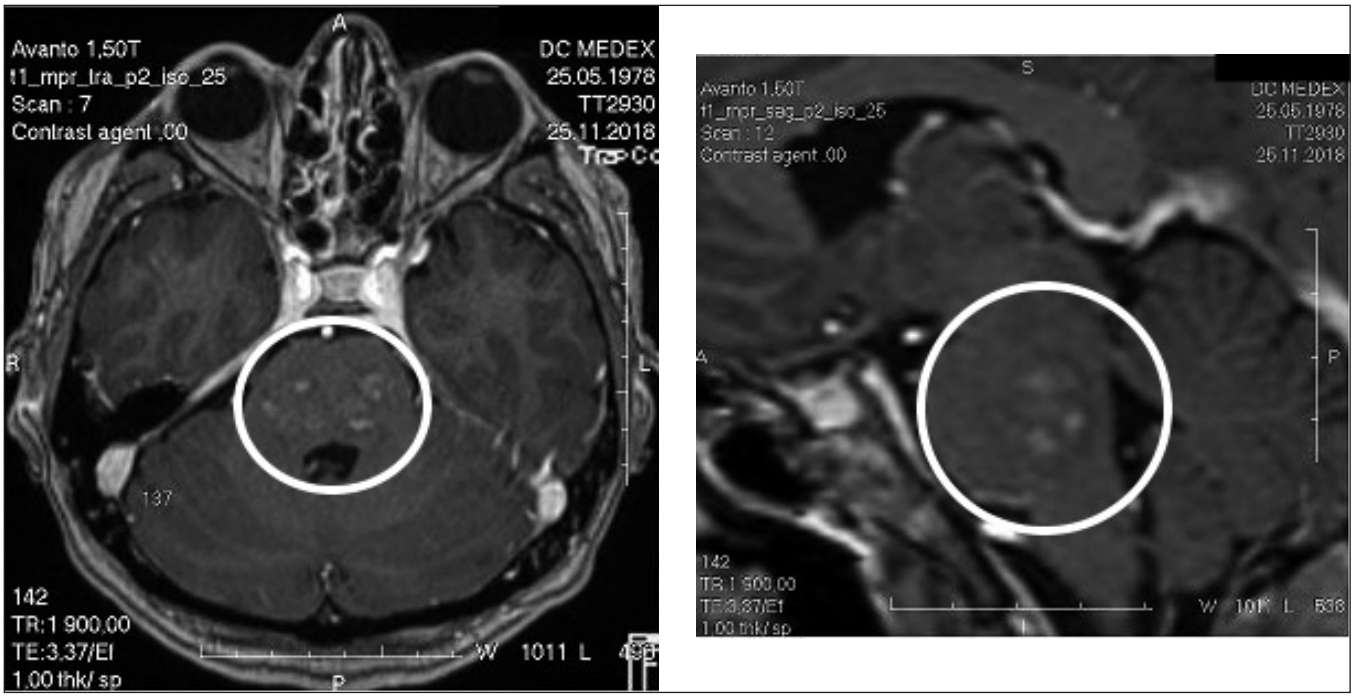

Fig. 1. MR images at the post-gadolinium scans, heterogeneous accumulation of contrast in the structure of the indicated region is preserved.

with complaints of sustained dizziness, vertigo, unsteady gait, blurred vision, weakness, fatigue, and decreased performance for more than a month. He has no relevant medical or family history. General clinical examination was normal. Clinical neurologic examination showed diplopia when looking to the left, left-sided facial hypoesthesia, slight deviation of the tongue to the left, light tremor of the hands at complicated Bare-probe. Nery, Stryumpel, Sharapov, and Chaddok symptoms were positive on one or both sides. Meningeal symptoms were not detected. He performed the coordination tests with intent, staggering in the Romberg pose.

A lumbar puncture was performed just on admission and CSF analysis revealed lymphocytic pleocytosis of 16 cell/μl (lymphocytes) with normal protein and glucose. Viral serology for HSV1/2, CMV, EBV, VZV, mumps, adenovirus, and enterovirus was negative. CSF PCR was reported to be negative for DNA of herpesviruses, *M.tuberculosis*, and *Tox. Gondii*. The oligoclonal band also was not revealed.

MR imaging showed a site of the small infratentorial lesions of an inflammatory nature in the pons with spreading to the upper and lower cerebellar peduncles without clear contours, a region of an amplified at T2 W1 and weakened at T1 W1 signal has a total size of 22x47x28 mm, at the post-gadolinium scans, heterogeneous accumulation of contrast in the structure of the indicated region is preserved (Figure 1 and Figure 2).

The patient did not have a brainstem biopsy for neuropathological diagnosis but met the clinical and MRI criteria of CLIPPERS as proposed by Tobin et al. [6], and an extensive workup did not yield an alternative diagnosis. Oral steroid treatment was started with 12 mg of methylprednisolone per day i/v, tapered to 4 mg of methylprednisolone per day during a month, and accompanied with a variety of steroid-sparing agents. We observed marked improvement of the clinical symptoms and partial resolution of MRI lesions after the use of methylprednisolone.

DISCUSSION

CLIPPERS Syndrome is chronic inflammation with a disorder of the pons, MR imaging of the contrast enhancement of the perivascular spaces in the pons, which responsive to steroids. The disease is extremely rare, its etiology and pathogenesis remain unknown. Although a full set of diagnostic criteria has not yet been determined MRI finding showing punctate enhancement in a "pepper-like appearance" centred on the pons is regarded as the hallmark of CLIPPERS [1, 6]. However, new data show involvement in the inflammatory process of remote areas of the brain and spinal cord, which reduces the diagnostic value of neuroimaging findings [4]. Besides, such clinical and neuroimaging findings can occur at other pathologies and therefore cannot be considered quite specific [7, 8]. The diagnostic value of the second important criterion, response to steroids, seems even more controversial. Indeed, almost all diseases with a pronounced inflammatory component respond to immunosuppressive drugs, including steroids. Not surprising, corticosteroid therapy was typically associated with the disappearance of neurologic symptoms and regression of post gadolinium contrast enhancement [6]. So, successful steroid therapy is the crucial criterion to confirm ex juvantibus the diagnosis of CLIPPERS. However, the clinical effect of steroids is usually temporary and rare radical. Disease progression is accompanied by a reduction in remissions and a weakening effect. The final component of the differential diagnostic scheme, the absence of other alternative causes,

Fig. 2. MR images of an inflammatory nature disorders in the pons with spreading to the upper and lower cerebellar peduncles without clear contours, a region of an amplified at T2 W1 signal has a total size of 22x47x28 mm.

seems to be the most important. Indeed, some patients initially diagnosed with CLIPPERS syndrome because of the characteristic clinical and radiological symptoms later turned out to be an atypical presentation of malignant lymphoma and other inflammatory and no inflammatory disorders [2, 9]. So, the exclusion of other possible causes of the identified abnormalities is a necessary condition for a correct diagnosis.

CONCLUSIONS

CLIPPERS syndrome appears to be a mysterious and uncommon variant of encephalitis involving inflammation of the brain parenchyma and vasculitis, which represent a real diagnostic challenge for health practitioners. Despite the efforts to clarify its pathogenesis, CLIPPERS syndrome remains a controversial nosological entity. It is thus necessary to evoke the diagnosis in front of any encephalitis etiologic

reason of which does not prove, particularly if recurrent and in elderly. Early diagnosis and adequate management can improve the prognosis of this disease. However, the low specificity and ambiguity of the proposed diagnostic symptoms do not allow to confidently differentiate CLIPPERS, which makes the search for new diagnostic tools and symptoms highly relevant.

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CASE STUDY

CLINICAL CASE OF EDWARDSIELLOSIS IN UKRAINE

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ABSTRACT

Edwardsiellosis is a zoonotic infectious disease that caused by Edwardsiella tarda and characterized by gastroenteritis in humans. Contaminated water can also be a source of infection. Primary nutritional toxicity clinical signs may mask aquatic zoonosis caused by *Edwardsiella tarda*. Infectious border control should ensure that the import into Ukraine of such preservatives of food products, highly probably infected by this agent is not allowed.

KEY WORDS: Edwardsiella tarda, gastroenteritis, African male, exotic disease, Ukraine

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INTRODUCTION

In Ukraine, not a single clinical case of Edwardsiellosis has been described yet. Edwardsiellosis is a zoonotic infectious disease that caused by *Edwardsiella tarda* and characterized by gastroenteritis in humans [1-3]. It has also been implicated in meningitis, biliary tract infections, peritonitis, liver and intra-abdominal abscesses, wound infections and septicaemia [4-10]. It has been often isolated from catfish fillets in processing plants and can spread to man via the oral route or a penetrating wound [11]. Contaminated water can also be a source of infection.

CLINICAL CASE

A student from Ghana, 22 years old, on the 3rd day of illness was hospitalized to the infectious disease unit of the Municipal Institution "Chernivtsi Regional Clinical Hospital" in Chernivtsi. Diagnosis on admission: food bacterial poisoning of moderate severity. Complaints – cramping abdominal pain, nausea, fever, fluid feces up to 5 times per day, general weakness. He associated his malaise with eating the seafood soup, which he has cooked by himself from a package given by his brother from Ghana.

An objective examination: body temperature 37.8 °C, pulse 92 beats per min, regular. AP 100/60 mm Hg. Tones of the heart are sound. Tongue is wet, furred. The abdomen is soft, rumbling, and painful along the intestine, mostly in the right iliac section. Stool is liquid without pathological admixture, badly smelling. He was consulted by a surgeon who had ruled out anyacute surgical pathology.

The next day the patient's condition began to improve: body temperature was normalized, abdominal pain decreased, but general weakness, poor appetite still remained. After 2 days the patient's condition was satisfactory, bowel movements were normalized, abdominal pain disappeared.

The patient has undergone general-clinical and bacteriological examination of feces. In the general analysis of blood, the shift of the leucocyte formula to the left was detected by increasing the cell-nuclear neutrophils to 26%. *Edwardsiella tarda* is isolated in the bacteriological culture method from feces.

While in hospital, the patient received the following treatment: a Trisolum solution, Rheosorbilact, Rehidron, Nifuroxazide, Norfloxacin, Enterosgel, Spasmalgon.

In the hospital, the patient spent five bed-days, and discharged in satisfactory condition with clinical recovery.

Edwardsiella tarda is a motile, facultatively anaerobic, Gram-negative rod that is categorized as a member of the family Enterobacteriaceae. The genus *Edwardsiella* was first recognized by Trabulsi et al in 1962, followed by a description of *E. tarda* in the mid-1960s. These organisms have successively been named the "Bartholomew group" by King and Adler, the "Asakusa group" by Sakazaki and "Edwardsiella" by Ewing et al.

The genus *Edwardsiella* comprises a genetically distinct taxon weakly related to other members of the Enterobacteriaceae. It consists of bacteria differing strongly in their biochemical and physiological features, natural habitats, and pathogenic properties. The most common species of the genus is E. tarda, which was already described in 1965. Although it has been recovered from a variety of environmental and animal sources, E. tarda is predominantly found in freshwater and fish. Humans are regarded to be occasional hosts but are prone to serious diseases due to this organism. Most frequently, *E. tarda* causes gastroenteritis presenting as acute watery diarrhea resembling that produced by other toxigenic enteropathogens, but dysentery-like courses also occur. Immunocompromised patients, older adults, and children are predominantly affected. Extraintestinal infections such as septicemia - with a mortality rate near 50% - and wound

infections have also been reported. Exceptionally, *E. tarda* has also been found to cause meningitis, peritonitis, osteomyelitis, and liver abscesses. In 1980, a second *Edwardsiella* species was proposed by Grimont et al. and was named *E. hoshinae*. In contrast to *E. tarda, E. hoshinae* is found in relatively few ecological niches (i.e., birds, reptiles, and water). Although *E. hoshinae* has been isolated from human feces, its role as a human or animal pathogen has not been established. The third *Edwardsiella* species was created in 1981 and was called *E. ictaluri. E. ictaluri* shows unusual properties: Apart from having a low optimal growth temperature, this organism has been predominantly isolated from channel catfish, in which it causes fatal systemic infections known as enteric septicemia. Human infections due to *E. ictaluri* are not known; however, virulence-associated properties such as serum resistance, indicating the potential to cause human disease, have been documented for all *Edwardsiella* species.

E. tarda is typically isolated from fresh or brackish water environments such as river mouths. It has also been isolated from the intestines of humans (after eating fresh water food sources such as catfish or eels) and from animals, including reptiles and freshwater fish.

Of the three recognized species, only *Edwardsiella tarda* has been demonstrated to be pathogenic for humans. Chief infections associated with this species include bacterial gastroenteritis, wound infections such as cellulitis or gas gangrene associated with trauma to mucosal surfaces, and systemic disease such as septicemia, meningitis, cholecystitis, and osteomyelitis. Risk factors that are associated with *E. tarda* infections include exposure to aquatic environments or exotic animals (e.g., reptiles or amphibia), preexisting liver disease, conditions leading to iron overload, and dietary habits (e.g., raw fish ingestion). Although studies indicate that this bacterium is susceptible to most commonly prescribed antibiotics, fatal gastrointestinal and extraintestinal infections have been described.

The source and reservoir are fresh and salty waters, fish, reptiles. The path of infection of people is water and food, a predominant risk factor – consuming raw or underexposed fish, intestines that are contaminated.

The main clinical manifestations (according to the described 72 cases are bacterial gastroenteritis, bacteremia caused by *Edwardsiella*, meningitis, cholecystitis, osteomyelitis, hepatic abscess). Fatal gastrointestinal and extra-intestinal forms are described. Case fatality rate is 44.6-61.1%. Concomitant liver cirrhosis is determined as an independent risk factor associated with a fatal outcome [12].

Pathogenic bacteria may have virulence genes that are absent in nonpathogenic bacteria, making them virulent. Virulence genes may also be present in both pathogenic and nonpathogenic bacteria but may be functional only in pathogenic ones. It has been reported that seven genes, named *orfA*, *fimA*, *gadD*, *katB*, *mukF*, *ssrB*, and *gyrB* were specific to pathogenic *E. tarda* [13, 14].

CONCLUSIONS

This clinical case of rare zoonotic infectious disease one more time proved that there is no borders for infection as general pathogenic concept. Migration and globalization of infectious pathology is a new reality. Classical bacteriological investigation of patient with gastrointestinal disorder is necessary for the obtaining of pure culture and final confirmation of etiologic diagnosis. Attentive epidemic history taking is essential in the formation of hypothesis according to presumptive clinical diagnosis. For the purpose of general prevention of this disease, it is a strongly recommended to conduct a sufficient heat treatment when seafood consumed. Primary nutritional toxicity clinical signs may mask aquatic zoonosis caused by *Edwardsiella tarda*. Infectious border control should ensure that the import into Ukraine of such preservatives of food products, highly probably infected by this agent is not allowed.

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CASE STUDY

SIMULTANEOUS HIATAL HERNIA PLASTICS WITH FUNDOPLICATION, LAPAROSCOPIC CHOLECYSTECTOMY AND UMBILICAL HERNIA REPAIR

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ABSTRACT

The article presents a case report of patients with multimorbid pathology – hiatal hernia with gastroesophageal reflux disease, cholecystolithiasis and umbilical hernia. Simultaneous surgery was performed in all cases – laparoscopic hiatal hernia with fundoplication, laparoscopic cholecystectomy and umbilical hernia alloplasty (in three cases – by IPOM (intraperitoneal onlay mesh) method and in one – hybrid alloplasty – open access with laparoscopic imaging). After the operation in one case there was an infiltrate of the trocar wound, in one case – hyperthermia, which were eliminated by conservative methods. The follow-up result showed no hernia recurrences and clinical manifestations of gastroesophageal reflux disease.

KEY WORDS: hiatal hernia, cholecystolithiasis, umbilical hernia, simultaneous operation

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INTRODUCION

Present-day possibilities of endovideoscopic technologies allow us to carry out a wide range of surgical interventions on the organs of the abdominal cavity, extraperitoneal space, and the anterior abdominal wall. The opportunities for simultaneous operations (operations for two or more diseases during single anesthesia) exist along with the improvement of operative techniques, gaining experience in endoscopic surgery [1]. In particular, laparoscopic cholecystectomy (LCE) for cholecystolithiasis is performed simultaneously with umbilical plastics [2, 3], postoperative [4] or hiatal hernia [5].

CASES REPORT

In this report, we present clinical observations of patients with combined pathology, who underwent simultaneous laparoscopic plastic surgery of hiatal and umbilical hernias and LCE (table 1).

A common feature of all patients (case 1-4) was a long history of recurrent pain in the right upper abdomen, dyspeptic syndrome (heartburn, belching, heaviness after eating, etc.), episodic administration of antacids, H_2-histamine blocker inhibitors or proton pump inhibitors with short-term effect. In all cases, the examination revealed asymptomatic umbilical hernia, and ultrasound examination of the abdominal cavity – cholecystolithiasis. Due to the dyspeptic symptoms, an endoscopic examination detected signs of gastroesophageal reflux, and later, according to the results of computed tomography, a hiatal hernia of type 1 or 2 by SAGES was diagnosed [6, 7]. In addition, increase of the BMI, in case 1, 2, 4 – concomitant arterial hypertension and / or heart failure were found in all cases.

Main indications for surgical intervention were umbilical hernia and cholecystolithiasis, but due to the presence of symptomatic hiatal hernia (symptoms of GERD and the ineffectiveness of conservative therapy), additional intervention – laparoscopic hiatal hernioplasty and fundoplication were offered to the patients.

The operations were performed under combined anesthesia (epidural anesthesia at the level of Th 6-8 and tracheal intubation, mechanical ventilation with inhalation anesthesia). An incision of the skin up to 10 mm is made on the border of the middle and lower third of the line from Xiphoid Processus to the umbilicus at the beginning of the procedure. A carboxyperitoneum up to 12–14 mm Hg is created by the Veresh needle and a 10-mm trocar for laparoscopic optics is inserted. Three additional 5 mm trocars are inserted: the first – at the Desjardins point, the second – in the area 4–5 cm below the costal arch on the left along the mid-clavicular line, the third – 1–2 cm below the left costal arch on the anterior clavicle-axillary line. The Nathanson retractor is inserted 2 cm below Xiphoid Processus and 1 cm to the left of the midline to fix the left lobe of the liver.

During the first stage of the intervention, the stomach and the abdominal esophageal portion are mobilized

Table 1. General characteristics of patients.

Indicator	Case 1	Case 2	Case 3	Case 4
Sex	female	female	female	male
Age, year	55	51	61	57
Cholecystolithiasis	+	+	+	+
Hiatal hernia, SAGES type	1	1	2	1
GERD	+	+	+	+
Umbilical hernia, size	< 2 cm	< 2 cm	>2 cm	>2 cm
BMI	26.2	28.3	28.1	25.4
Comorbidities:				
AH	+	–	+	+
HF	–	–	+	–

Note. SAGES – Society of American Gastrointestinal and Endoscopic Surgeons; GERD – gastroesophageal reflux disease; BMI – body mass index; AH – arterial hypertension; HF – heart failure.

Table 2. Features of surgical interventions and their result

Indicator	Case 1	Case 2	Case 3	Case 4
1st stage:				
- posterior crurography	+	+	+	+
- alloplasty	–	+	–	+
- fundoplication	Nissen	Nissen	Toupet	Nissen
- anterior crurography	–	+	–	–
Duration of the 1st stage (min)	45	115	50	40
2nd stage:				
- LCE	+	+	+	+
Duration of the 2nd stage (min)	10	10	20	15
3rd stage: umbilical hernia repair:				
- IPOM	–	–	+	+
- hybrid	+	+	–	–
Duration of the 3rd stage (min)	15	15	10	15
The total duration of the operation (min)	100	170	110	105
Postoperative complications:				
- trocar wound infiltrate;	–	+	–	–
- postoperative hyperthermia	+	–	–	–
Duration of inpatient treatment, days	7	8	7	6
Long-term result (from 2 years):				
- recurrence of umbilical hernia	–	–	–	–
- symptoms of GERD	–	–	–	–

along a large curvature, using the 5-mm Ligasure, and a 5-mm ultrasound dissector lowered in the proximal direction. A Laparoscopic Goldfinger Retractor is inserted into the area of the His-angle for the esophageal traction. A gastric tube size 28-30 Fr is inserted transorally. The posterior crurorаphy is performed with atraumatic suture material – Ethibond 2-0 or V-lock 90. In one case, the posterior crurorаphy and anterior crurorаphy was performed due to the atypical diaphragm's legs configuration. Antireflux cuff is created by Nissen or Toupet method with fixation by Ethibond 2-0 sutures with the anterior esophageal wall. In one case, the diaphragm defect was closed with an allograft Crurasoft V-shaped mesh prosthesis with Polytetrafluoroethylene (PTFE) coating.

A standard LCE is performed in the second stage of the operation. A 5-mm trocar is inserted 1-2 cm below the right costal arch along the anterior-axillary line towards the gallbladder, additionally. A 10-mm trocar is inserted instead of Nathanson's retractor in the direction of the liver at an angle of 45°. After identification of the Kalo triangle, the vesical duct is clipped with three titanium clips or Hem-o-lok clips, the vesical artery is clipped with a titanium clip and dissected ,using laparoscopic scissors or by bipolar coagulation. A step-by-step subserous cholecystectomy is performed from the "neck". The gallbladder is removed from the abdominal cavity through a trocar incision after the hemostasis in the area of the gallbladder bed.

The IPOM umbilical hernia repair is performed in the third stage. A 10-mm trocar for optics in the left

megostric area along the mid-clavicular line is inserted, and a 5-mm trocar – in the iliac region on the left along the mid-clavicular line. The hernia sac together with the peritoneum is mobilized. Through a 10-mm trocar, a composite mesh graft based on Sepra technology is inserted into the abdominal cavity, which is fixed with a herniostepler along the perimeter and in the area of the hernia gate.

A primary 10-mm trocar for laparoscopic optics is placed below umbilical ring, and in the last stage of the operation, a hybrid umbilical hernia is performed – the hernia gate is separated from the peritoneum in one case. A round mesh graft with stiffening ribs, coated with a hydrogel or has a PTFE coating, is inserted into the abdominal cavity through the hernia gate. There are two polypropylene tapes in the center of the allograft, taken out of the hernia gate and fixed to the aponeurosis of the rectus abdominal muscles by separate sutures, fixing the allograft according to the type "in lay".

A common feature of surgical interventions in all cases was the necessity to change the position of the patient on the operating table and the location of the endovideosurgical console, and the operating team. To correct hiatal hernia and GERD, the console was located to the left of the patient, who was in the anti-Trendelenburg position with a roller under the thoracic spine. The surgical team is located as follows: the surgeon stands between the lower extremities, assistants – to the right and left of the patient. To perform LCE: the position of the patient is with a tilt to the left. The location of the operating team changes: the surgeon – to the left of the patient, the first assistant between the lower extremities, the second assistant to the right of the patient. To perform IPOM, the endovideosurgical console is located to the right of the patient. The patient's position is inclined to the right. This should be taken into account when planning the operation and securely fix the patient to the operating table.

General characteristics of surgical interventions and their result are given in table. 2.

Thus, frequency of simultaneous operations for comorbidities with existing indications for surgical treatment is steadily increasing, improving laparoscopic methods. These clinical observations demonstrate the possibility of surgical correction of four diseases simultaneously – hiatal hernia, GERD, cholecystolithiasis and umbilical hernia.

A similar case was described by T. Yamanaka et al. [8]. In one case they performed a laparoscopic hiatal hernia repair with Nissen fundoplication, LCE and open umbilical hernia repair.

In contrast, in all our cases we performed hiatal hernia repair with fundoplication and LCE, but for umbilical hernia we performed allogeneic plastics, using mesh graft by IPOM method in three cases. The hernia sac was openly removed from paraumbilical incision in one case (hybrid plastics), used for the introduction of a 10-mm trocar with subsequent alloplasty, applying an endoprosthesis of a special configuration for the umbilical ring. There were no significant postoperative complications. Recurrence of umbilical hernia and manifestations of GERD during a long-term observation period was not found.

Besides, these observations demonstrate the possibility of similar combinations. This should be taken into consideration when examining and planning surgical treatment. T. Yamanaka et al. believe that such a combination is a variant of the incomplete Saint's triad [8].

CONCLUSIONS

In our opinion, the Saint's triad, multiple hernias and many other comorbidities, including cholecystolithiasis, have a common pathogenetic mechanism. This mechanism is connective tissue disorders resulting in systemic and local disorders of collagen metabolism [9-11]. This peculiarity explains coexistence of several diseases in one patient and confirms the necessity for a comprehensive analysis of clinical symptoms and additional research methods. The allogeneic hernioplasty of all localization can be considered as an optimal surgical intervention in case of the connective tissue disorders multiple manifestations.

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